



US007745148B2

(12) **United States Patent**
Duan et al.

(10) **Patent No.:** **US 7,745,148 B2**
(45) **Date of Patent:** **Jun. 29, 2010**

(54) **RANDOM HOMOZYGOUS GENE
PERTURBATION TO ENHANCE ANTIBODY
PRODUCTION**

2005/0026246 A1* 2/2005 Sale et al. 435/69.1
2006/0240021 A1 10/2006 Li et al.

OTHER PUBLICATIONS

(75) Inventors: **Roxanne Duan**, Bethesda, MD (US);
Michael Goldblatt, McLean, VA (US)

Sui et al. *Virology* XXX(2009)XXX-XXX; eline version.*
Mao et al, *Virology*, 2009, 6:154-167.*

(73) Assignee: **Functional Genetics, Inc.**, Gaithersburg,
MD (US)

International Search Report dated Dec. 22, 2008, received in Inter-
national Application No. PCT/US07/82972.

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 120 days.

Gumienny, et al., "CED-12/ELMO, a Novel Member of the CrkII/
Dock180/Rac Pathway, Is Required for Phagocytosis and Cell
Migration", *Cell*, vol. 107, pp. 27-41 (Oct. 5, 2001).

(21) Appl. No.: **11/928,393**

Kohler, et al., "Continuous cultures of fused cell secreting antibody of
predefined specificity", *Nature*, vol. 256, pp. 495-497 (Aug. 7, 1975)
(Abstract).

(22) Filed: **Oct. 30, 2007**

Limin Li, et al., "tsg101: A Novel Tumor Susceptibility Gene Isolated
by Controlled Homozygous Functional Knockout of Allelic Loci in
Mammalian Cells", *Cell*, vol. 85, pp. 319-329 (May 3, 1996).

(65) **Prior Publication Data**

* cited by examiner

US 2009/0136927 A1 May 28, 2009

Primary Examiner—Misook Yu

Related U.S. Application Data

Assistant Examiner—Mark Halvorson

(60) Provisional application No. 60/855,127, filed on Oct.
30, 2006.

(74) *Attorney, Agent, or Firm*—Steven B. Kelber; Berenato &
White, LLP

(51) **Int. Cl.**
G01N 33/53 (2006.01)

(57) **ABSTRACT**

(52) **U.S. Cl.** **435/7.1; 435/451**

The invention reflects enhanced antibody expression of an
antibody of interest by cell lines transformed by random
homozygous gene perturbation methods to either increase or
decrease the expression pattern of a gene of the cell line other
than the antibody of interest. The transformed cell line exhib-
its specific productivity rates, SPR, for the RHGP trans-
formed cell lines of 1.5 or more, as compared with the anti-
body expressing cell line parents prior to transformation by
RHGP. A knock out or anti-sense construct may be devised to
reduce expression of the target gene, a promoter may be
inserted to enhance expression of the target gene. The anti-
bodies expressed by the transformed cell lines exhibit the
binding properties of their parent cell lines prior to transfor-
mation with RHGP, and increase Total Volumetric Production
of said antibody by said cells in a given volume.

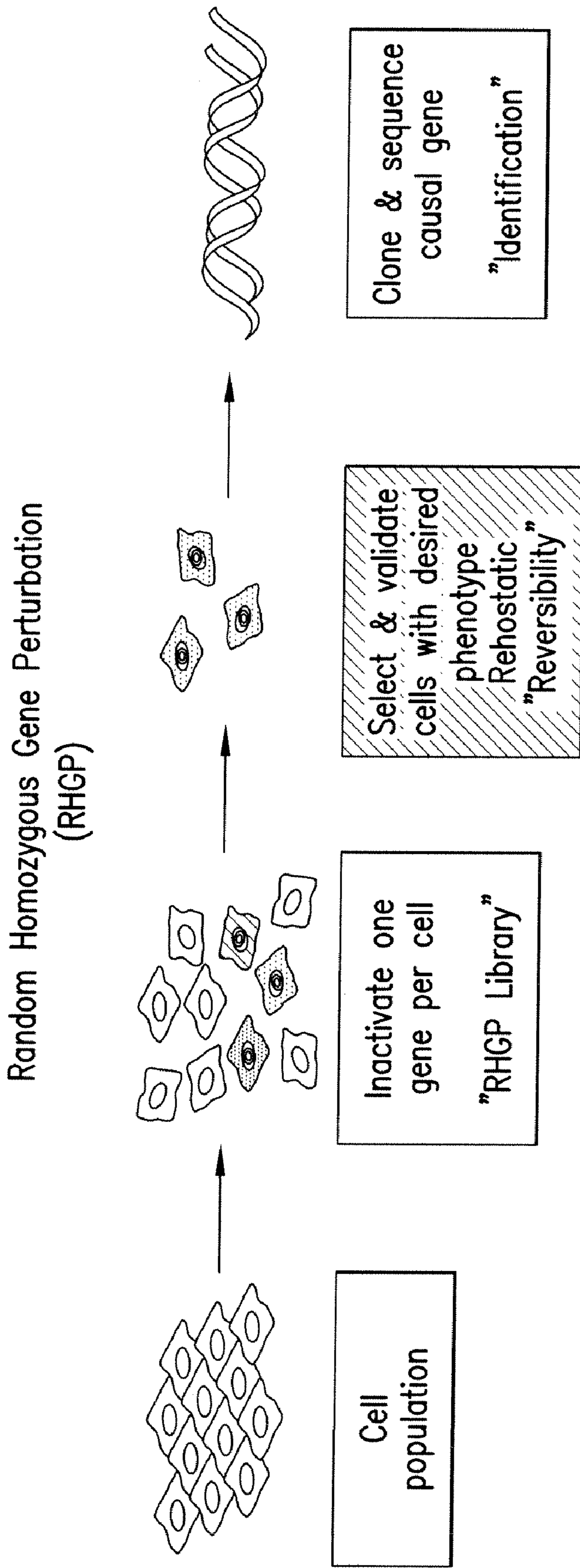
(58) **Field of Classification Search** None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,816,567 A 3/1989 Cabilly et al.
5,679,523 A * 10/1997 Li et al. 435/6
5,981,214 A 11/1999 Skoultchi
6,054,561 A 4/2000 Ring
6,835,816 B2 12/2004 Cohen et al.
7,479,379 B2 * 1/2009 Ryczyn et al. 435/69.1
2003/0208784 A1 11/2003 Ruley et al.
2004/0197866 A1 10/2004 Johnson et al.
2004/0241808 A1 12/2004 Pasqualini et al.

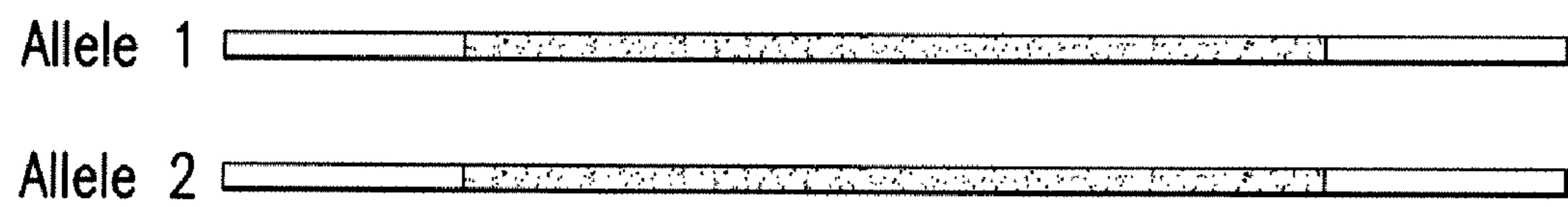
8 Claims, 19 Drawing Sheets



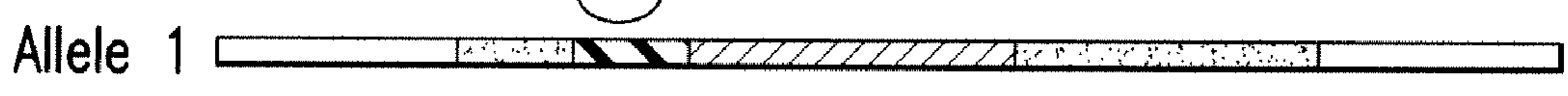
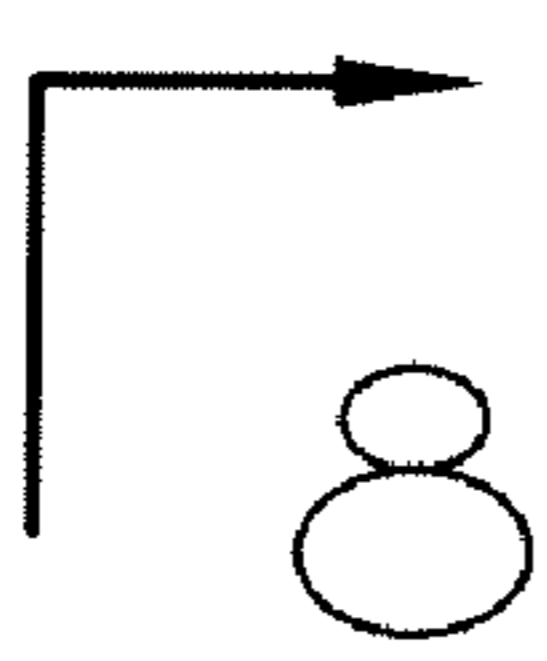
- RHGP links gene identity with functionality
- RHGP enables simultaneous target discovery and validation
- RHGP enables unbiased genome wide knockout or over expression within a short time

FIG.1

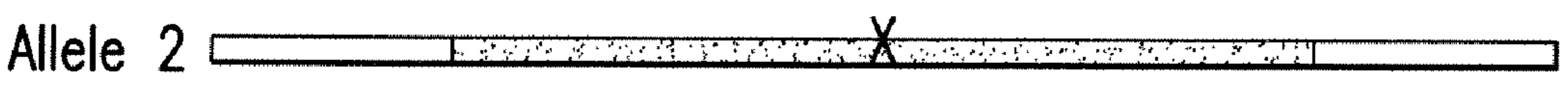
Disruption of Cellular Genes by Random Homozygous Gene Perturbation (RHGP)



Selection Marker
Transactivator



Regulated Antisense
Expression



Antisense Message blocks
expression of second allele

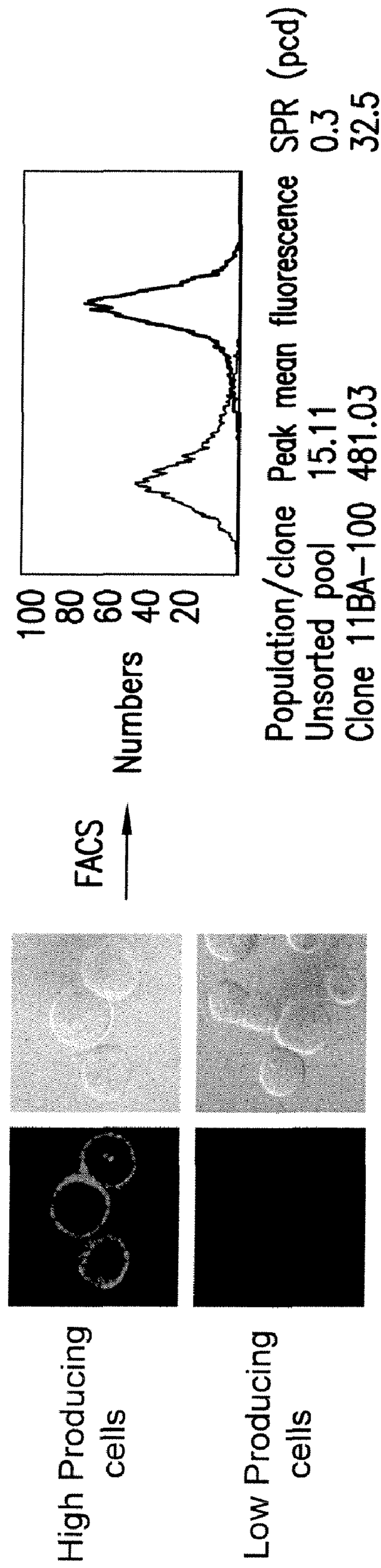
FIG. 2

Assay Systems for Finding High Producers

Rationale:

Level of cell surface associated IgG is
directly proportional
to level of secreted IgG

Step 1. HTP Screening based on cell-surface associated IgG



Step 2. Confirmation ELISA assay for secreted IgG

FIG. 3

Selecting High Antibody Producers by FACS

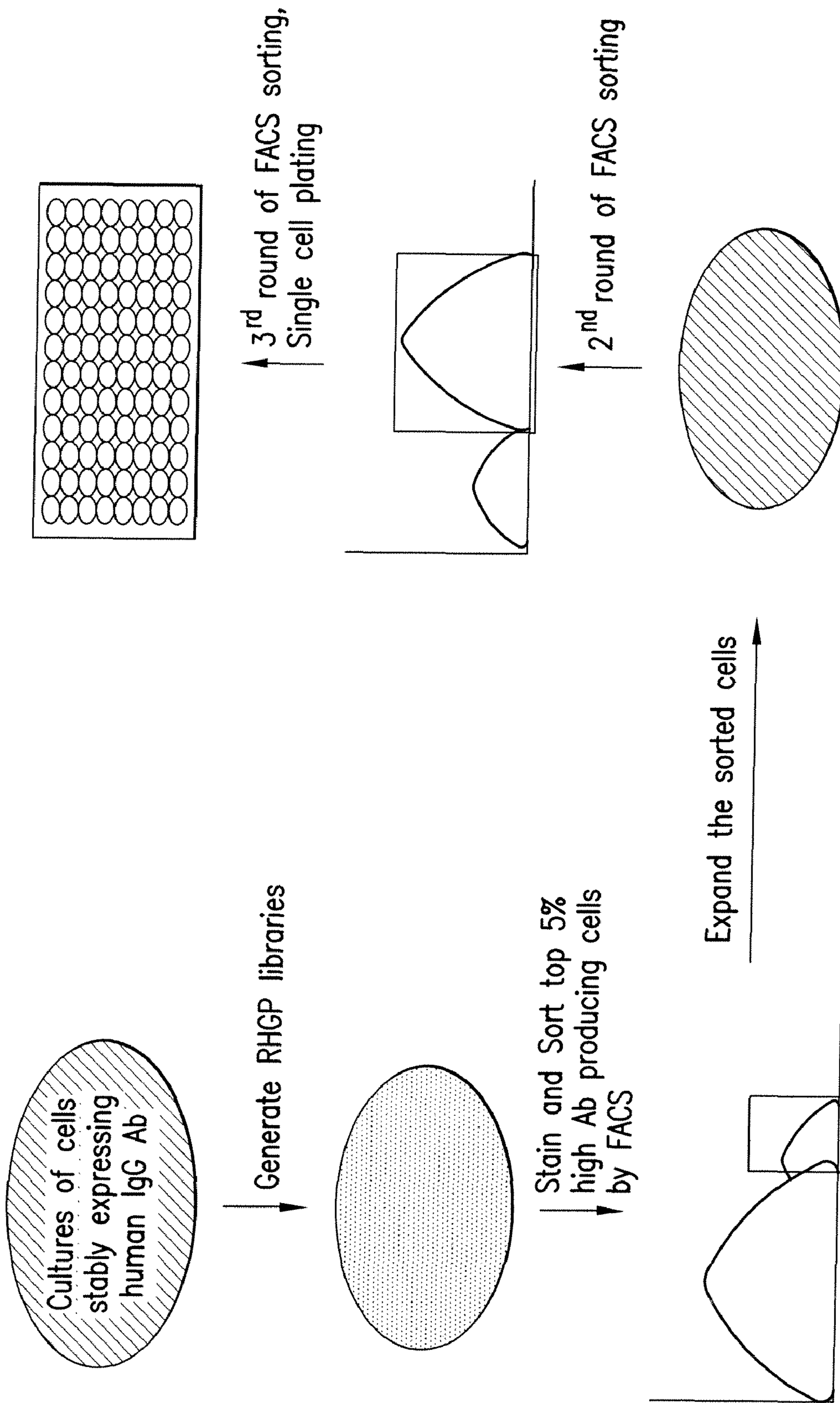


FIG.4

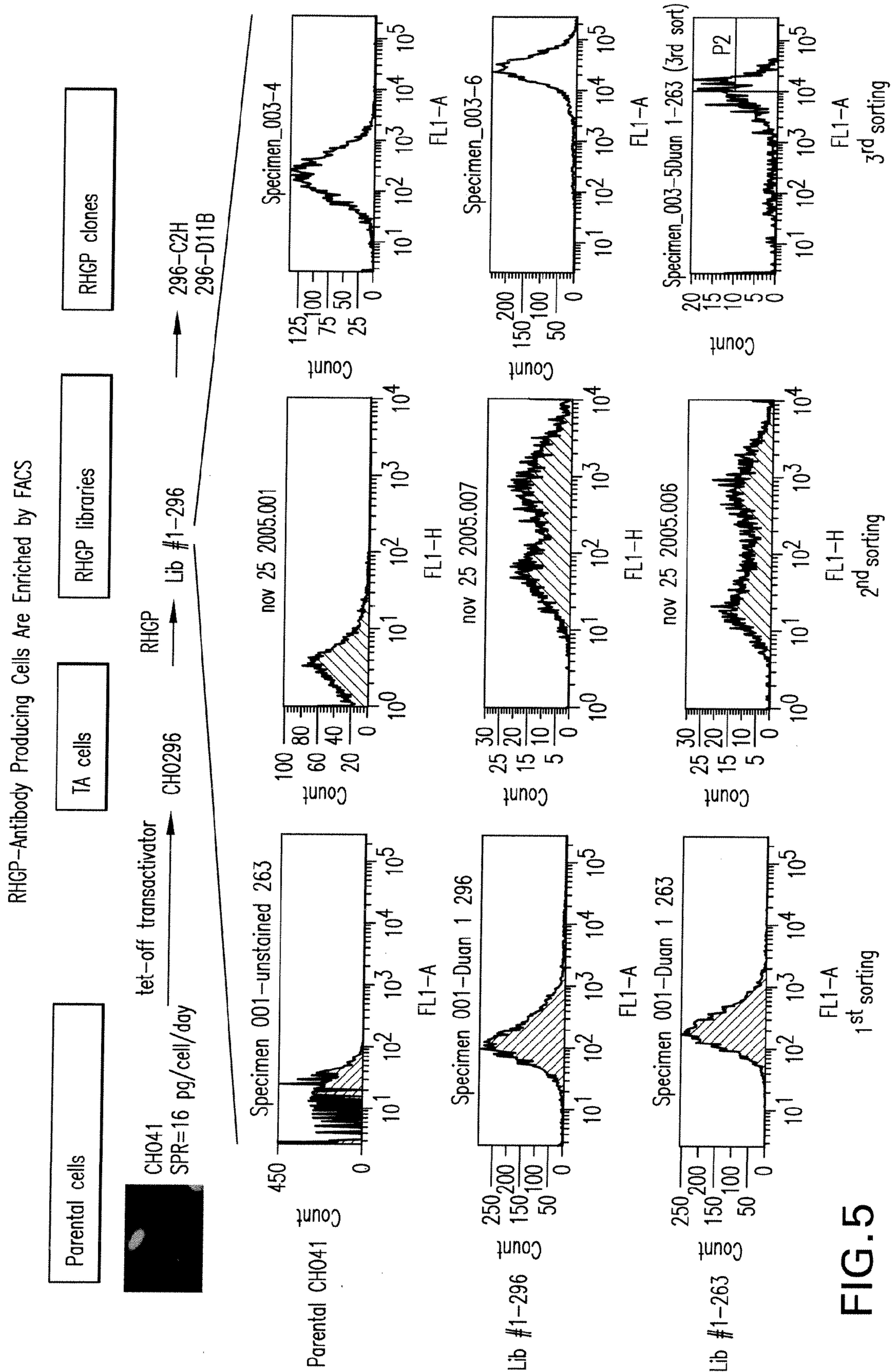
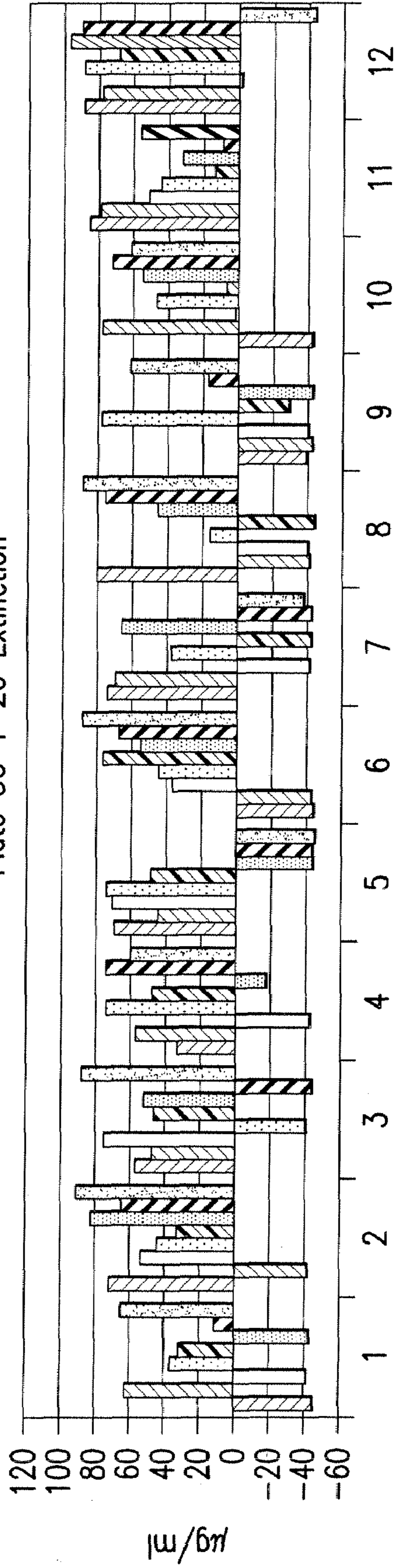


FIG. 5

Total Productivity Measurements to Select for High Producers

Plate 5C 1-26 Extinction



We can first screen hundreds of clones using an 'Extinction' experiment that reflects total productivity, then identify cells with high specific productivity (SPR).

In general, total productivity in this kind of experiment correlates with SPR.

FIG.6

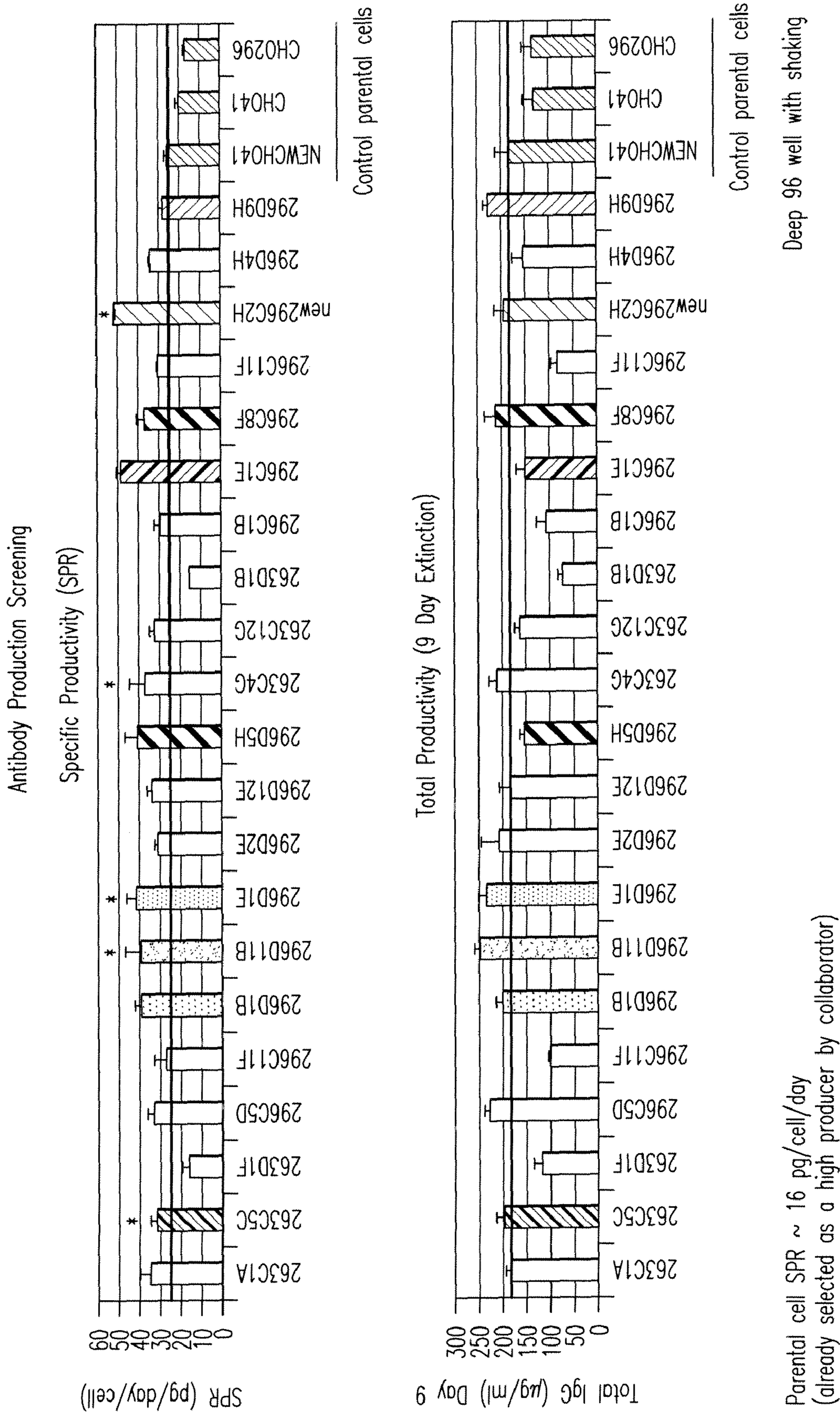


FIG. 7

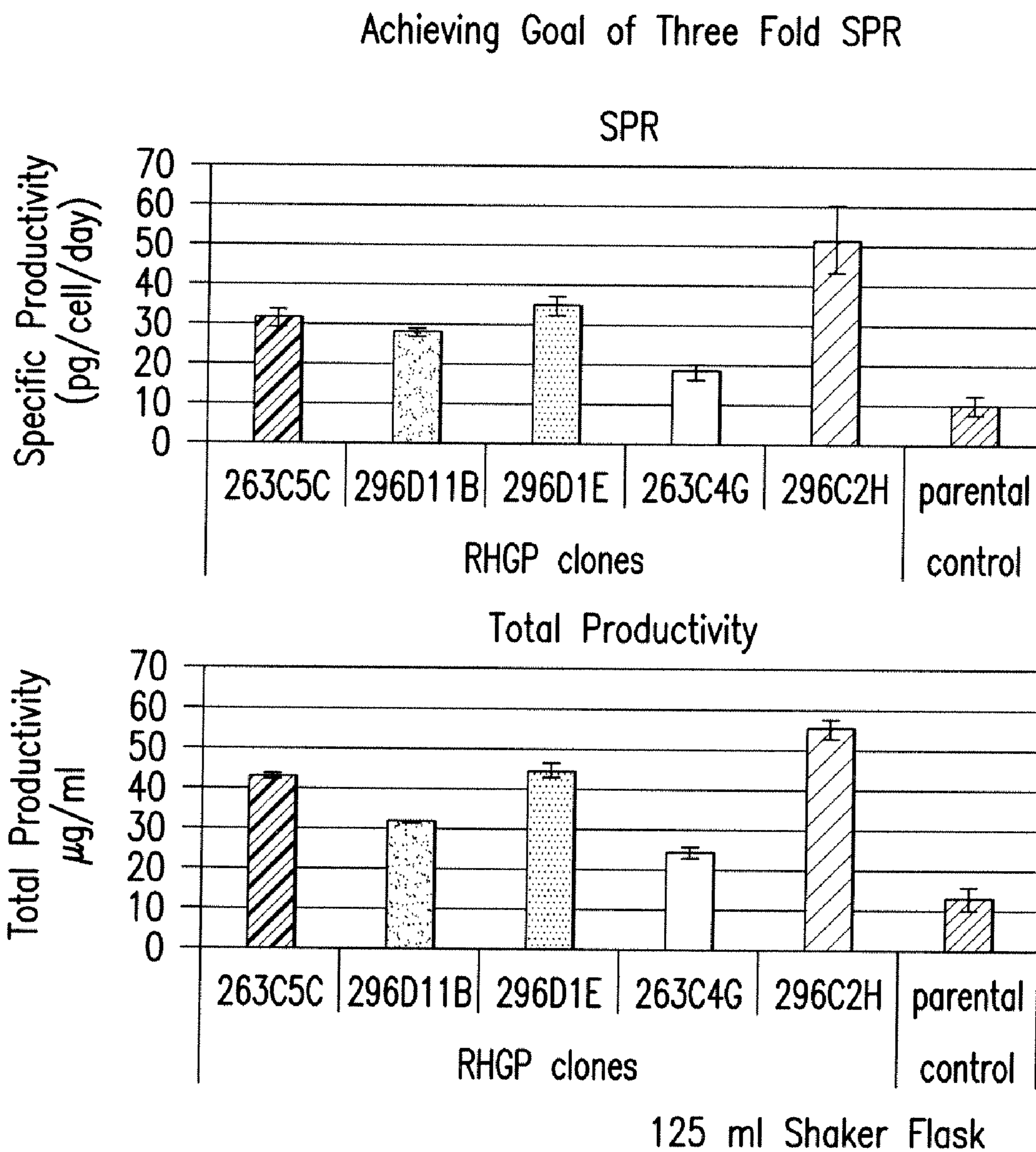
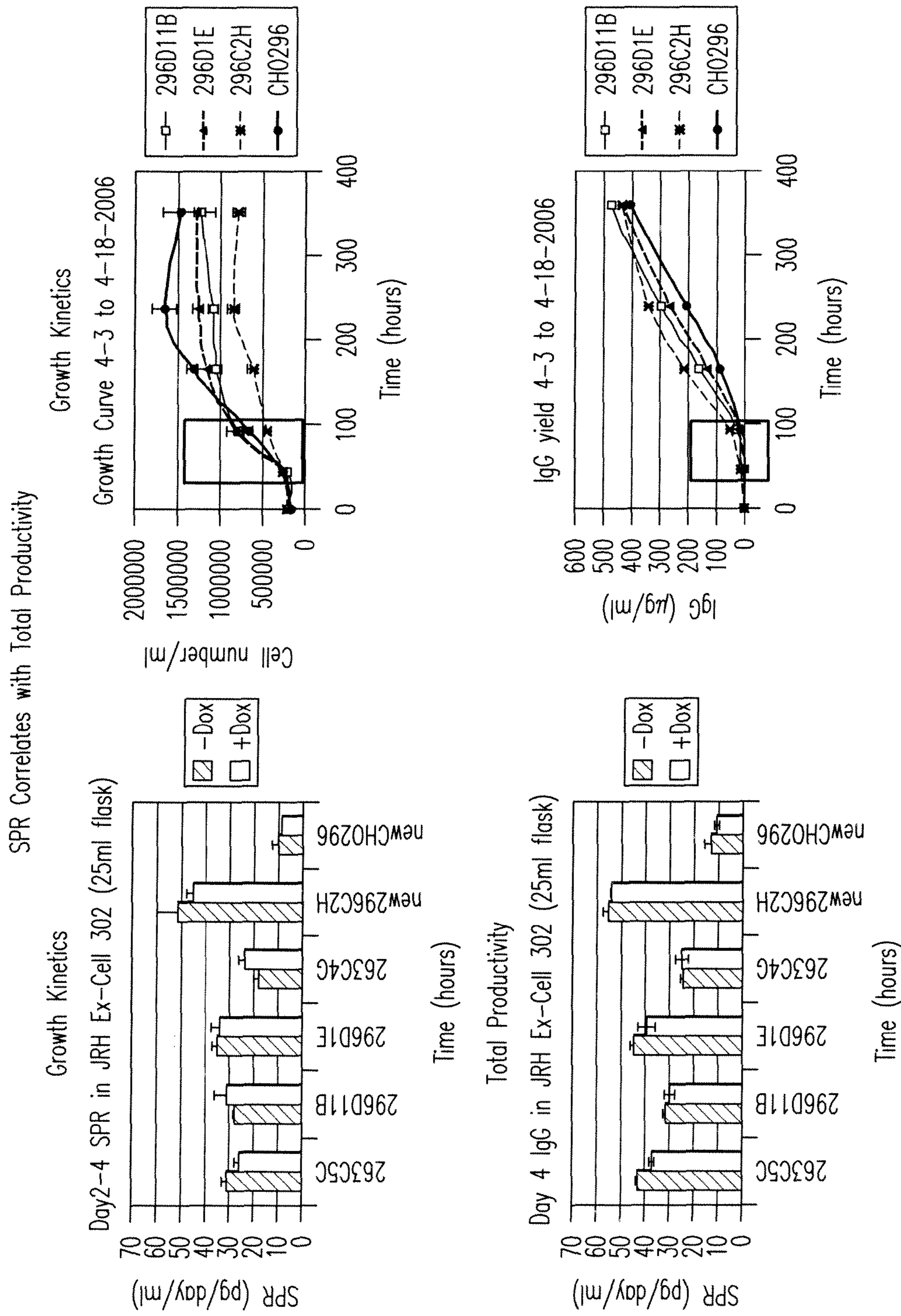


FIG.8



1. SPR and Total Productivity correlate on Day 4-7 with each other in this rich media. On Day 10-15, IgG production start to reach saturation.
2. SPR and TP are relatively high in our RHKO clones.
3. Growth kinetics are important in choosing the best clone.

FIG. 9

Quality Control:
Antigen Binding Capabilities of High Producer mAbs
Are Similar to That of Original mAb

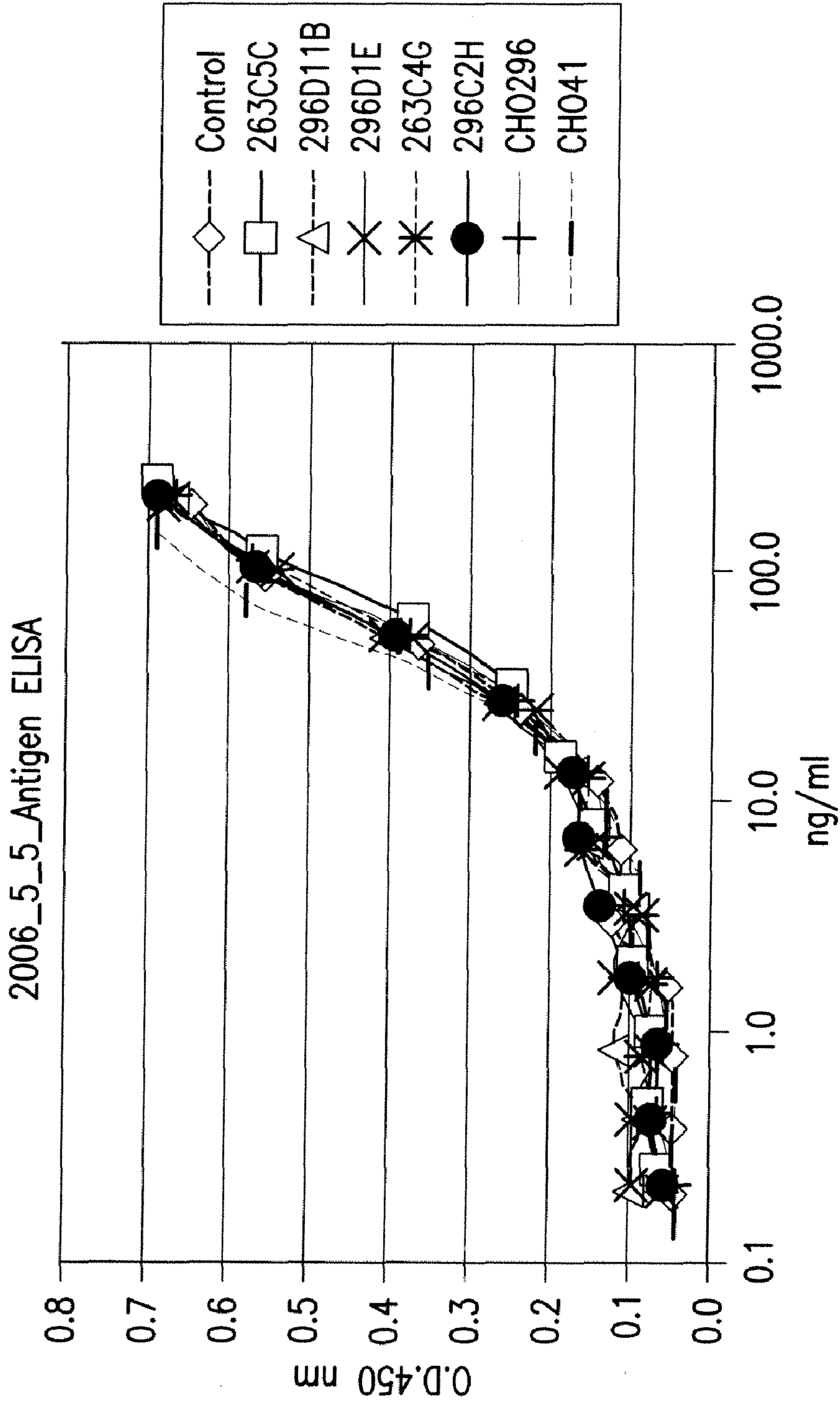
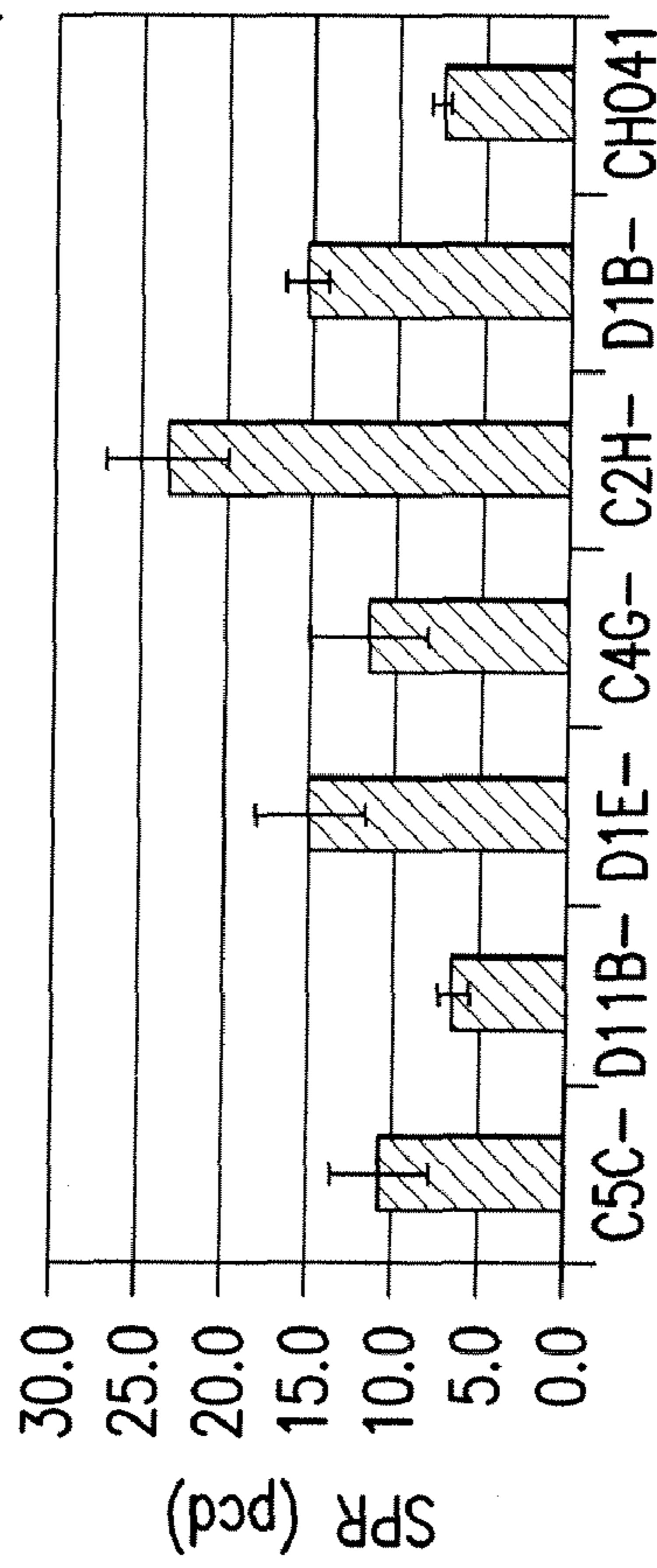


FIG.10

Long Term (8 week) Stability of RHGP-Clones

SPR for RHKO clones cultured in antibiotics-free Ex-Cell media for long term stability test (7-31 to 9-13-2006)



SPR of RHKO clones passed in selection media from 7-31 to 9-13-06

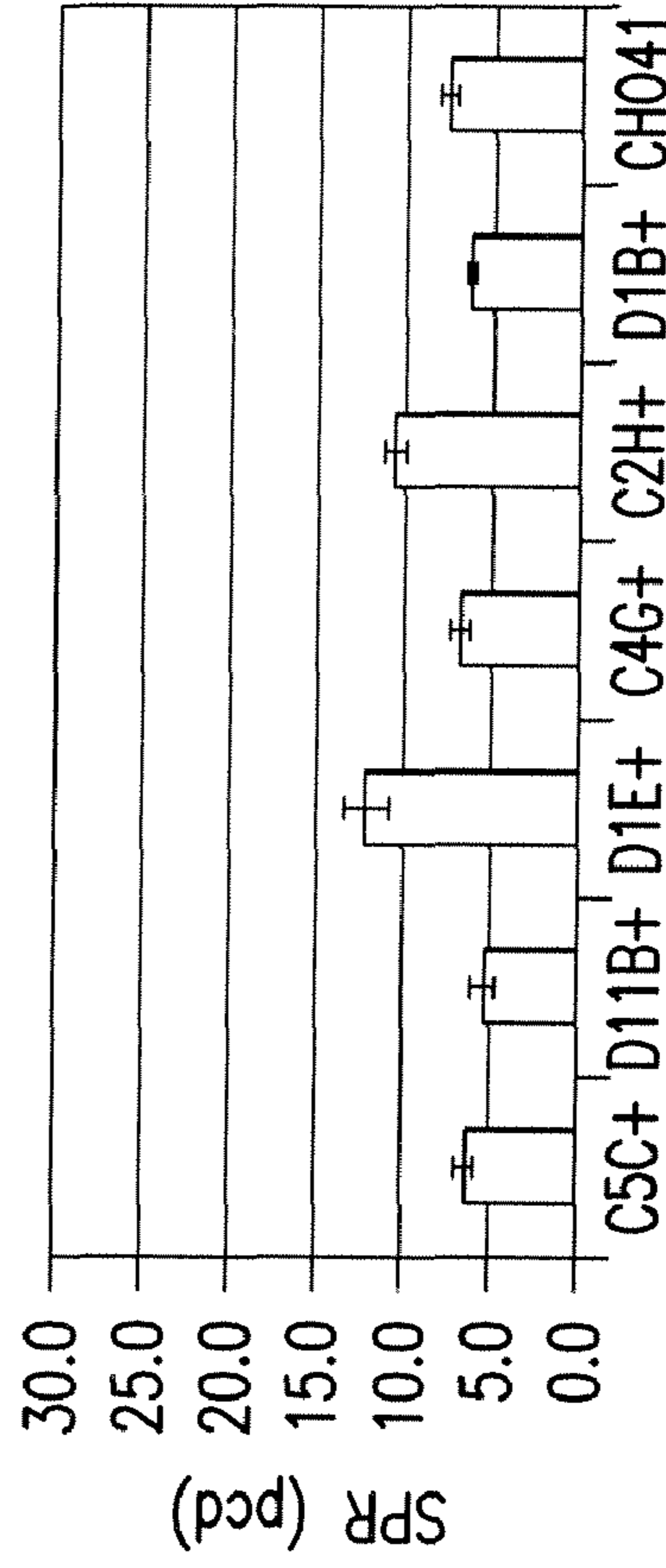
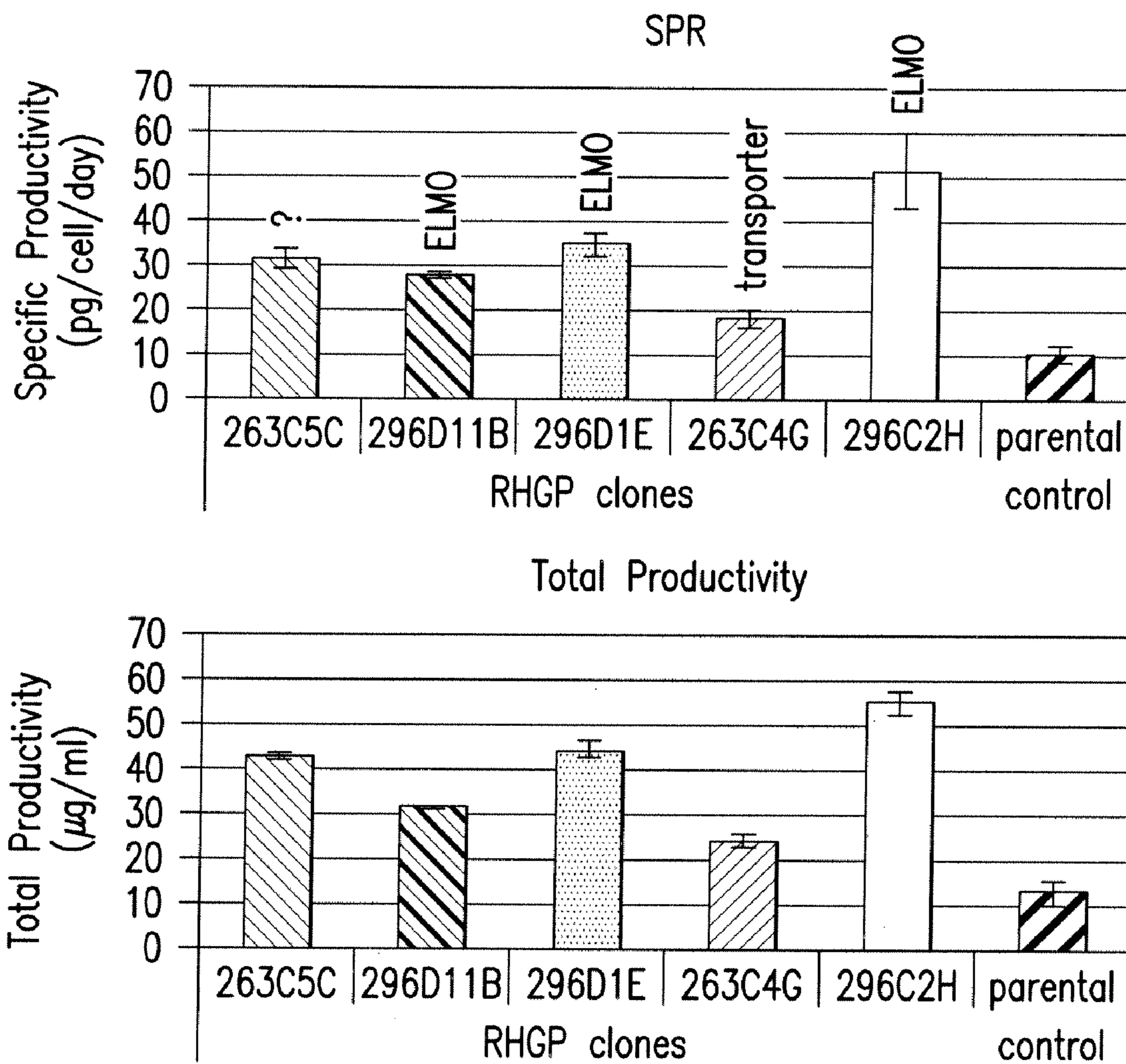


FIG. 11

Higher SPR Found in a Number of Clones



125 ml Shaker Flask

FIG. 12

ELM01
First RHGP Gene Identified from High Specific Productivity Clones

Sequence View: Difference Format, Color behind non-matches

Human ELM01	1	mpppadivkvaiewpgaypkImeidqkpl	saiikevcdgwslanheyfa	lqhadssnfyiteknrneikngti	lrlttspaqnagg	l	h
Mouse ELM01	1	S
Rat ELM01	1	S
CHO-ELM01	1
Human ELM01	91	riqssmdak	lea	lkd	las	l	srdv
Mouse ELM01	91
Rat ELM01	91
CHO-ELM01	91
Human ELM01	181	ikkiasfvnksaidisi	l	qrs	l	a	iesm
Mouse ELM01	181
Rat ELM01	181
CHO-ELM01	181
Human ELM01	271	lrsiil	th	vira	qra	innemahg	ly
Mouse ELM01	271
Rat ELM01	271
CHO-ELM01	271

FIG. 13A

Human ELM01	361	f	i	n	h	v	n	p	a	m	d	f	t	q	t	p	p	g	m	l	a	l	d	n	m	l	y	f	a	k	h	h	q	d	a	y	i	r	i	v	l	e	n	s	s	r	e	d	k	h	e	c	p	f	r	s	s	i	e	l	t	k	m	l	c	e	i	l	k	v	g	e	l	p	s	e	t	c	n	d	f	h	p	m	f	f	t	h	d	r
Mouse ELM01	361																																																																																								
Rat ELM01	361																																																																																								
CHO-ELM01	361																																																																																								
Human ELM01	451	s	f	e	e	f	f	c	i	c	i	q	l	l	n	k	t	w	k	e	m	r	a	t	s	e	d	f	n	k	v	m	q	v	v	q	e	m	r	a	l	t	t	k	p	s	l	d	q	f	k	s	l	q	n	l	s	y	e	i	l	k	i	r	q	s	e	r	m	n	g	e	d	f	q	s	r	p	i	e	l	k	e	k	i					
Mouse ELM01	451																																																																																								
Rat ELM01	451																																																																																								
CHO-ELM01	451																																																																																								
Human ELM01	541	q	p	e	i	l	e	l	i	k	q	q	r	l	n	r	l	v	e	g	t	c	f	r	k	l	n	a	r	r	r	q	k	f	w	y	c	r	l	s	p	n	h	k	v	l	h	y	g	d	l	e	e	s	p	q	e	v	p	h	d	s	l	q	d	k	l	p	v	a	d	i	k	a	v	v	t	g	k	d	c	p	h	m	k	e	k	g		
Mouse ELM01	541																																																																																								
Rat ELM01	541																																																																																								
CHO-ELM01	541																																																																																								
Human ELM01	631	l	k	q	n	k	e	v	l	e	l	a	f	s	i	l	y	d	s	n	c	q	l	n	f	i	a	p	d	k	h	e	y	c	i	w	t	d	g	l	n	a	l	l	g	k	d	m	s	d	l	t	r	n	d	l	l	s	m	e	i	k	l	r	l	d	l	e	n	i	q	i	p	d	a	p	p	p	i	p	k	e	p	s	n					
Mouse ELM01	631																																																																																								
Rat ELM01	631																																																																																								
CHO-ELM01	631																																																																																								
Human ELM01	721	d	f	v	y	d	c	n																																																																																		
Mouse ELM01	721																																																																																								
Rat ELM01	721																																																																																								
CHO-ELM01	721																																																																																								

FIG. 13B

Vector Map of pLlexp-Antisense ELM01

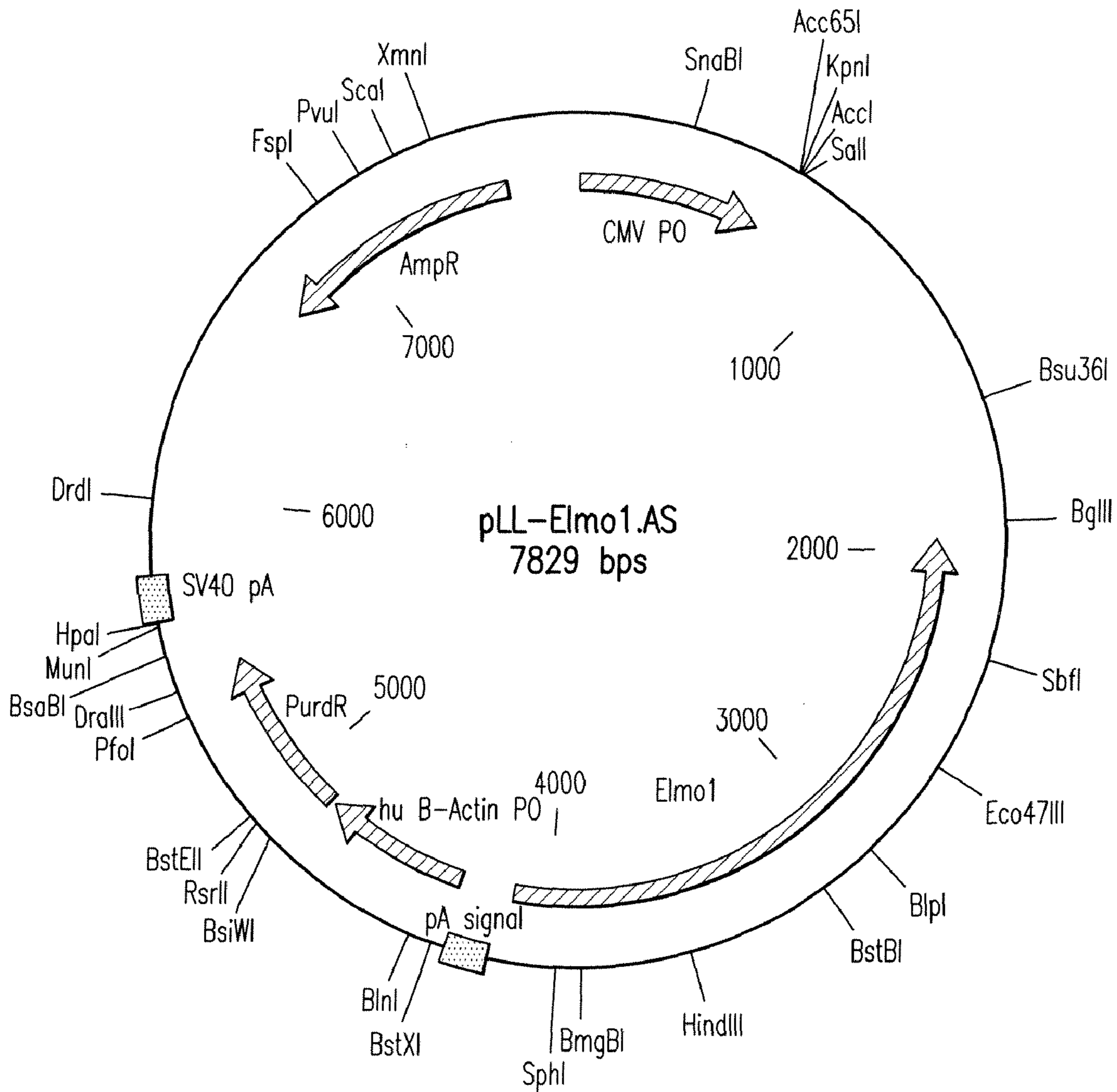


FIG. 14

Elmo Protein is Down Regulated
RHGP Clones with High Specific Productivity Rate

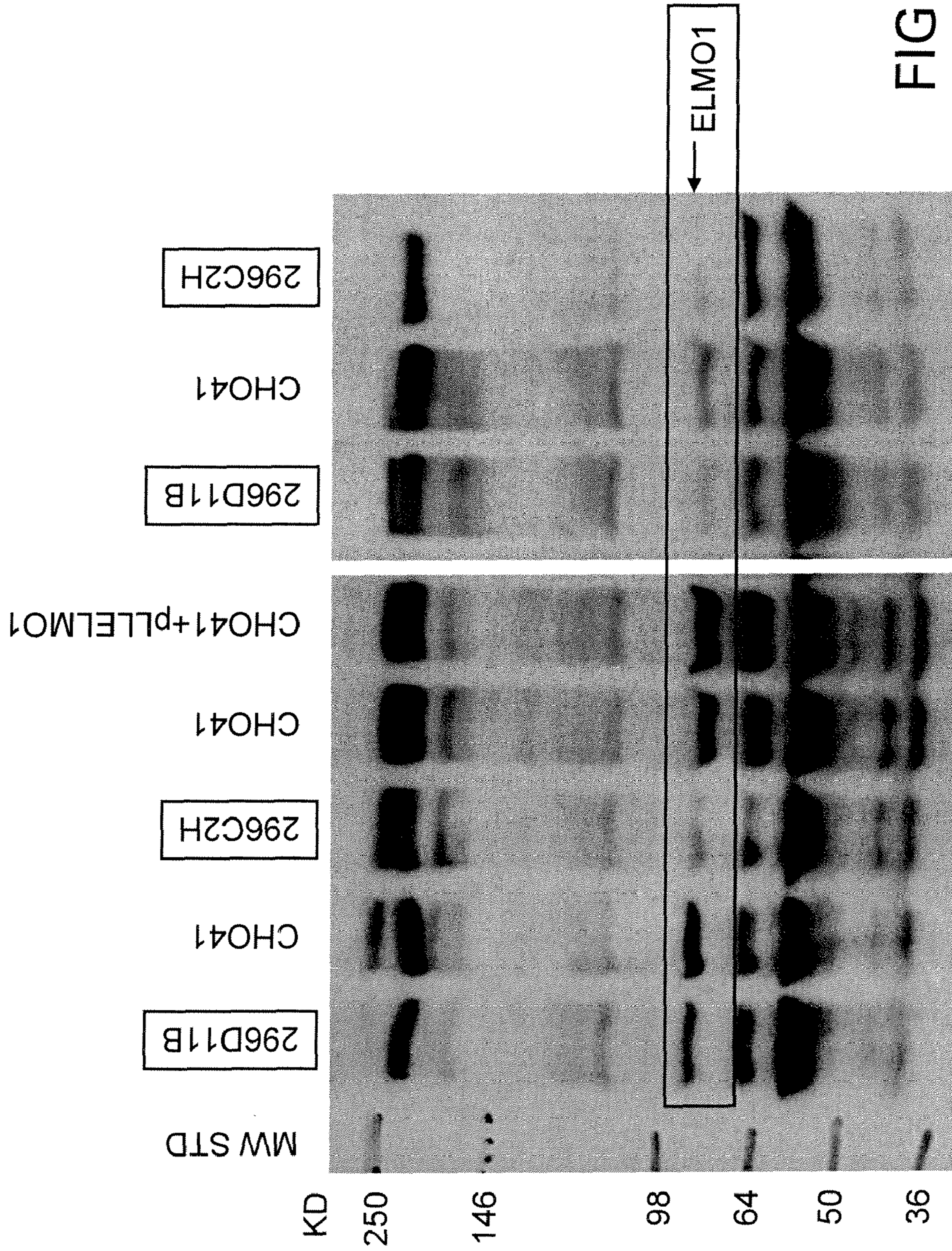


FIG.15

Expression of ELMO-1 Antisense in CHO41 Resulted in Increased SPR but Only Slight Down-regulation of ELMO Protein

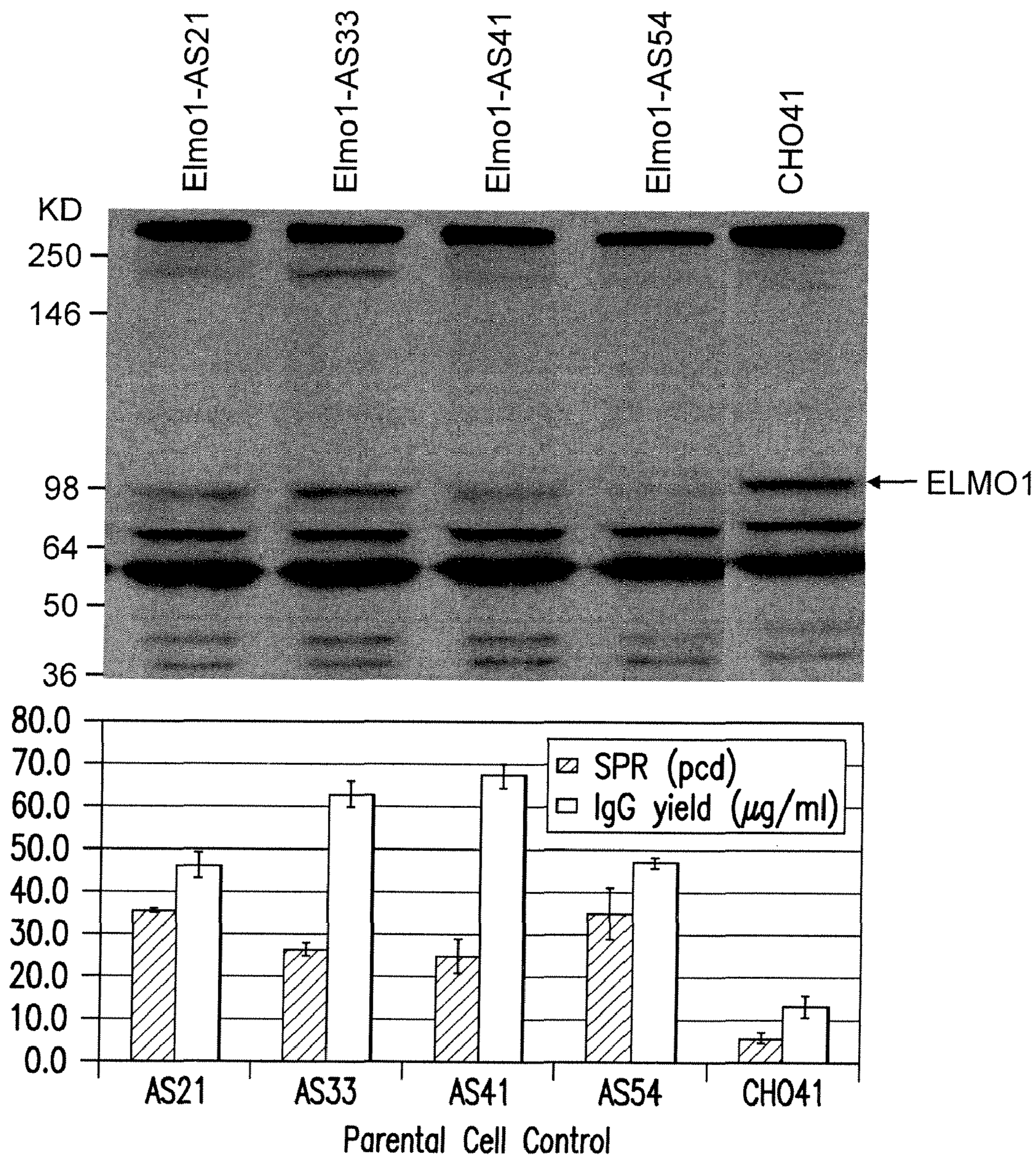


FIG. 16

Alignment of Ion Transporter Protein Sequences
Between CHO, Mouse, Rat and Human

Rat Transpor	1	mafgnlacivcklhfisktdndvls	g	cgllgdpcpaqrppgi	lrrpgcpsr	pcvpgg	gdgarlega	lrrsgaagw	lrargfrerpks	-----											
Mouse transp		-----		-----		-----		-----		-----											
CHO ITP Prot		-----		-----		-----		-----		-----											
human transp		-----		-----		-----		-----		-----											
Rat Transpor	91	vedlltrgrprlqhnahrcpqqpes	q	rgsgsssaasaqqrase	lggrrpgr	gadepr	l	rartssarrpgr	garvtw	gpdspgpe											
Mouse transp		-----		-----		-----		-----		-----											
CHO ITP Prot		-----		-----		-----		-----		-----											
human transp		-----		-----		-----		-----		-----											
Rat Transpor	181	hslrpraallgggavpgrppssaa	l	yaplr	apralama	idrrreaa	gsgagr	qpapae	engslppg	daaasap	lggr	ragsgsae	iqplp								
Mouse transp	1	-----		-----		-----		-----		-----		-----									
CHO ITP Prot	1	-----		-----		-----		-----		-----		-----									
human transp		-----		-----		-----		-----		-----		-----									
Rat Transpor	271	alhpsggphssccaalaaapslll	l	ldydgsvlpf	l	gglgggyqkt	lvv	l	twipalfig	fsqfads	flldqpn	fwch	gagkgte	lagatvtg							
Mouse transp	55	-----		-----		-----		-----		-----		-----		-----							
CHO ITP Prot	55	-----		-----		-----		-----		-----		-----		-----							
human transp		-----		-----		-----		-----		-----		-----		-----							
Rat Transpor	361	rwgdmgnwtsp	satp	fsta	swgttsnr	snssdtpp	l	psppgkgn	ndsnc	chawdy	girtgl	l	qnv	skwd	l	vcd	h	akv	h	akfs	llvg
Mouse transp	145	-----		-----		-----		-----		-----		-----		-----		-----		-----		-----	
CHO ITP Prot	145	-----		-----		-----		-----		-----		-----		-----		-----		-----		-----	
human transp		-----		-----		-----		-----		-----		-----		-----		-----		-----		-----	

FIG.17A

Rat Transpor	451	lifgylitgciadwvrrpvllfsv	ifilifigltvalsvnvtmfstlrffegclagi	iltlyalrielpgkrf	itmvsvfamagq
Mouse transp	235	lifgylitgciadwvrrpvllfst	ifilifigltvalsvnvtmfstlrffegclagi	iltlyalrielpgkrf	itmvsvfamagq
CHO ITP Prot	235	lifgylitgciadwvrrpvllfsj	ifilifigltvalsvnvtmfstlrffegclagi	iltlyalrielpgkrf	itmvsvfamagq
human transp	1	-----	-----	-----	-----
Rat Transpor	541	fimpglaa]crdwqvlqaliicpflml	lywsifpes]rwlmatqqfesakllilyl	tqkncvspedesikgvmpeleke]srrpkkvciv	
Mouse transp	325	fimpglaa]crdwqvlqaliicpflml	lywsifpes]rwlmatqqfesakllilyl	tqkncvspedesikgvmpeleke]srrpkkvciv	
CHO ITP Prot	325	fimpglaa]crdwqvlqaliicpflml	lywsifpes]rwlmatqqfesakllilyl	tqkncvspedesikgvmpeleke]srrpkkvciv	
human transp	15	fimpglaa]crdwqvlqaliicpflml	lywsifpes]rwlmatqqfesakllilyl	hftqknrmpegdikgv]peleke]srrpkkvciv	
Rat Transpor	631	kvgtrnlwknivvlcvnsltgygihhcfarsmmghevkvpl	lenfyadytmasialasc]amc	lvvr]grrggll]fmi]t]al]asll	
Mouse transp	415	kvgtrnlwknivvlcvnsltgygihhcfarsmmghevkvpl	lenfyadytmasialasc]amc	lvvr]grrggll]fmi]t]al]asll	
CHO ITP Prot	415	kvgtrnlwknivvlcvnsltgygihhcfarsmmghevkvpl	lenfyadytmasialasc]amc	lvvr]grrggll]fmi]t]al]asll	
human transp	105	kvgtrnlwknivvlcvnsltgygihhcfarsmmghevkvpl	lenfyadytmasialasc]amc	lvvr]grrggll]fmi]t]al]asll	
Rat Transpor	721	qlgl]n]ligkysqhpdselq]k]lav	gmsdsvkdkfsiafsivgmfashavgs]svffcaeitptvir	cggl]g]v]lasagfgm]tapi]iel	
Mouse transp	505	qlgl]n]ligkysqhpdselq]k]lav	gmsdsvkdkfsiafsivgmfashavgs]svffcaeitptvir	cggl]g]v]lasagfgm]tapi]iel	
CHO ITP Prot	505	qlgl]n]ligkysqhpds	-----	gmsdsvkdkfsiafsivgmfashavgs]svffcaeitptvir	cggl]g]v]lasagfgm]tapi]iel
human transp	195	qlgl]n]ligkysqhpds	-----	gmsdsvkdkfsiafsivgmfashavgs]svffcaeitptvir	cggl]g]v]lasagfgm]tapi]iel
Rat Transpor	811	hnqkgyflhhiifaccl]i]c]i]l]l]pesrdqn]peni	angehytrqp]l]shkkgeqp]l]t]nae]k]dysg]l]hdvaavgdg]l]segatan		
Mouse transp	595	hnqkgyflhhiifaccl]i]c]i]l]l]pesrdqn]peni	angehytrqp]l]shkkgeqp]l]t]nae]k]dysg]l]hdvaavgdg]l]pegatan		
CHO ITP Prot	587	hnqkgyflhhiifaccl]i]c]i]l]l]pesrdqn]peni	angehytrqp]l]shkkgeqp]l]t]nae]k]dysg]l]hdvaavgdg]l]pegatan		
human transp	277	hnqkgyflhhiifaccl]i]c]i]l]l]pesrdqn]peni	angehytrqp]l]shkkgeqp]l]t]nae]k]dysg]l]hdvaavgdg]l]pegatan		
Rat Transpor	901	gmktm			
Mouse transp	685	gmktm			
CHO ITP Prot	677	gmksm			
human transp	367	gmkgm			

FIG. 17B

**RANDOM HOMOZYGOUS GENE
PERTURBATION TO ENHANCE ANTIBODY
PRODUCTION**

CROSS REFERENCE TO RELATED CASES

This application is a utility application claiming benefit of U.S. provisional application Ser. No. 60/855,127, filed Oct. 30, 2006, which is incorporated by reference in its entirety herein for all purposes.

STATEMENT REGARDING FEDERALLY
SPONSORED RESEARCH

This invention was made, in part, with U.S. government support under Defense Advanced Research Project Agency (DARPA) Agreement No. W91NF-050C0059. The United States Government may enjoy certain rights pursuant thereto.

BACKGROUND

1. Technical Field

The present invention relates to methods of altering cells to enhance production of proteins they have been raised to express. Particularly, this invention addresses the use of Random Homozygous Gene Perturbation to enhance antibody expression of an antibody-expressing host, by targeted insertion of DNA to either depress endogenous expression of a host protein, or enhance expression of a poorly expressed host protein, the change in expression being related to an increase in expression of the antibody expressed by the host cell.

2. Background of the Technology

Antibodies, particularly monoclonal antibodies, have become important biologic products both in mankind's arsenal against disease, and in research and development. While not the "magic bullet" once envisioned, more than a score of monoclonal antibodies, sometimes referred to as mAb, have been approved for therapeutic use. Just a few of these include the Trastuzumab antibody, the active agent in Herceptin® approved for the treatment of some breast cancers, Palivizumab, the mAb of Synagis® approved for the prevention/treatment of RSV, and Bevacizumab, a mAb present in Avastin®, approved for the treatment of colorectal cancer, and indicated to be effective in treating other conditions. Many more are known.

By contrast, there are literally thousands of antibodies, mAb and polyclonal, employed as workhorses in laboratories and research facilities around the world. Antibodies are useful as diagnostics, as agents to bind and isolate target molecules, to differentiate cells for testing, and other uses that take advantage of the specific binding properties of IgG to select out a single antigen, typically a biological molecule, bound or unbound, that may be of interest. Antibody production is fundamental business.

Methods of making antibodies are well established, although refinements are added constantly. The basic information was set forth as early as 1975, Kohler & Milstein, *Nature*, 256: 495-497 (1975). To prepare monoclonal antibodies, a host, typically a rabbit or the like, is injected with the antigen against which a mAb is sought. Following immunization, the spleen, and possibly lymph nodes, of the host are removed and separated into single cells. These cells are then exposed to the target antigen. Cells that express the desired mAb on their surface will bind to the immobilized antigen. These cells are cultured and grown, and fused with myeloma cells or other immortal cells to form hybridoma, which can be cultured to recover the expressed antibody.

Most antibodies, and virtually all therapeutic antibodies, need to be modified to avoid inducing a rejection reaction in a patient. The DNA encoding the antibody expressed by the hybridoma is isolated, and can be modified by the insertion or removal of bases, altered glycosylation profiles, and manipulation of framework regions and complementary determining regions, which affect the affinity and avidity with which the antibody binds to its target antigen. The resulting antibodies are humanized or "human" or otherwise modified (chimeric antibodies and veneered antibodies are common in the art). The state of the art as of about 1995 is reflected in U.S. Pat. No. 6,054,561, the relevant disclosure of which is incorporated herein by reference.

Once prepared and isolated, the DNA encoding the antibody may be transferred to a preferred mammalian cell line for expression in "production" or commercial amounts. It has long been recognized that Chinese Hamster Ovary cells (CHO cells) make excellent expression vehicles for recombinant or non-endogenous DNA. See U.S. Pat. No. 4,816,567. There has been developed a series of DHFR deficient CHO cell strains, which permit the amplification of inserted DNA encoding specific proteins or DNA sequences, as set forth in U.S. Pat. No. 5,981,214. This latter patent describes the use of homologous recombination to target a specific gene or expression region of a cell—in the case in question, to induce expression of a heterologous gene. Other suitable cell lines include 293HEK cells, HeLa cells, COS cells, NIH3T3 cells, Jurkat Cells., NSØ cells and HUVEC cells. Other mammalian cell lines suitable for the expression of recombinant proteins have been identified in the literature, and are equally suitable for use in the invention of this application.

Once stabilized, current methods to increase production of the valuable antibodies tend to focus on increases the total productivity, that is, high volumetric productivity, so that a given amount of cells produces a given amount of antibodies. These methods tend to focus on improving the methods and environments used to cultivate the cells, to enhance total antibody production. In general, antibody production of greater than about 1 g/L is required for an industrially competitive process. Individual CHO cells are typically expressing in the range of 10-15 pg/cell/day.

Homologous recombination has been used in many contexts since about 1985. It was originally employed as a "knock-out" tool, allowing the suppression of an expressed gene, to study the response of the modified cell. Subsequent procedures were developed to allow the silencing of target genes. The use of anti-sense knock out constructs using a random homozygous knock out method (RHKO) is described, e.g., in Li et al, *Cell* 85: 319-329 (1996). In U.S. Patent Publication 20060240021 (U.S. patent application Ser. No. 10/524,426 filed Aug. 18, 2003) the use of RHKO techniques is disclosed to identify the genes involved in rapamycin resistance. The entirety of that disclosure is incorporated herein by reference. The ability to insert a construct into one allele, identify the cells where that allele has been successfully modified by quick throughput searching, such as for example by FACS (fluorescence activated cell sorter) and similar methods makes this a superior technique for selective identification and modification of a cell's genome. U.S. Pat. No. 6,835,816, incorporated by reference herein discloses the use of this technique in conjunction with genes reflecting tumor susceptibility, including TSG101 genes.

Accordingly, it remains a goal of the industry to find a way to increase the expression of antibodies, particularly recombinantly prepared antibodies, from expression hosts like CHO cells, 293HEK cells, HeLa cells, COS cells, NIH3T3 cells, Jurkat Cells, NSØ cells and HUVEC cells. and others,

in a stable and reproducible fashion, using available techniques to modify the genome of the cell.

SUMMARY

The invention demonstrates that cells that are good expression vehicles for recombinant antibodies can be modified to increase the specific productivity rate (SPR) of antibody producing cells by a factor of 1.5, 2 or even 3 fold above the expression range capable of the cell without such modification. Thus, by selectively altering the expression profile of the cell, using knock out techniques (Random Homozygous Gene Perturbation or RHGP) or expression enhancement techniques by inserting expression promoters rather than anti-sense RNA or other expression suppression constructs, antibody production by the cell can be enhanced. Enhancement values of 3-fold or more, SPR, have been achieved by suppression of the expression of targeted proteins. Enhanced SPR leads to enhanced volume productivity, permitting commercial collection of mAb on a heretofore desired but not achieved basis.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic illustration of the process of the invention.

FIG. 2 is a schematic illustration of the modification of a cell line genome by random homozygous gene perturbation according to the invention.

FIG. 3 is an illustration of the assays that can be used to demonstrate enhanced antibody expression by cells transformed according to the invention.

FIG. 4 is an illustration of how the repeated use of FACS sorting assays can enable sequestration of the cells exhibiting the highest SPR for a given antibody through the invention.

FIG. 5 is a schematic demonstrating the SPR enrichment for cell lines transformed according to the invention using repeated FACS assays.

FIG. 6 is a graph showing the distribution of SPR for cells modified by RHGP as compared with parent expression values.

FIG. 7 is a graph comparing SPR and TPV for cells exhibiting enhanced SPR values following RHGP to reduce Elmo1 expression levels.

FIG. 8 is a graph showing 3-fold enhancement of SPR and TPV using the process of the invention.

FIG. 9 is a four part graph demonstrating correlation of SPR with TVP of cells transformed with RHGP according to the invention.

FIG. 10 is a graph demonstrating the similarity in binding properties of antibodies expressed by cells transformed by RHGP to exhibit higher SPR values with parent cells of the same cell line that did not undergo RHGP.

FIG. 11 is a graph demonstrating long term stability of CHO cell clones modified by RHGP to enhance antibody SPR.

FIG. 12 is a graph demonstrating the presence of elevated SPR and TVP by several clones of a CHO cell line obtained by RHGP-induced downregulation of Elmo1 expression.

FIG. 13 reflects the sequence for the Elmo1 gene of humans, mice, rats and as present in CHO cells transformed by the invention.

FIG. 14 is a vector map of the plasmid used to induce downregulation of the Elmo1 gene through RHGP according to the invention.

FIG. 15 is a blotting photomicrograph demonstrating downregulation of Elmo1 in cells exhibiting enhanced antibody production following transformation by RHGP.

FIG. 16 is a graph demonstrating the increase in SPR of cells modified by RHGP as compared with the decrease in expression of Elmo1.

FIG. 17 is a sequence comparison for the ion transporter protein of human, rat, mouse and CHO cell, a target for RHGP pursuant to the invention.

DETAILED DESCRIPTION

Applicants' invention resides in the discovery that the Specific Productivity Rate or value of anti-body producing cells can be enhanced by altering the expression profile of the cell's endogenous genome without altering the genomic sequence about the antibody itself. Thus, as noted above, it is possible to insert expression enhancers, amplifiable genes, and the like, proximate to, or with, the inserted heterologous DNA that expresses the mAb of interest. These methods have their limits. Applicant's invention lies in the discovery that by inserting a construct at a locus other than that which encodes the antibody itself, protein expression profiles may be altered, thereby increasing the SPR for the antibody. In many cases, this will involve introducing a knock-out construct . . . and insert encoding, for example, anti-sense RNA, to down regulate or suppress expression and even translation of a particular protein. In other situations, it will involve inserting an expression construct, or a construct involving an enhancer or promoter or some other activator that enhances expression of a non-mAb protein, which is implicated in the mAb synthesis pathway, and thus upregulates mAb expression.

This is conveniently affected, in one example, by insertion of an anti-sense knock-out construct that deactivates or inactivates an unrelated protein. Not all knock-out or down regulation will increase mAb expression. There does not appear to be at this time a way to map the proteins whose expression profile can be affected in a way to predict whether that alteration will increase SPR of a given cell. Predictably, there are some proteins whose expression cannot be significantly downregulated without adversely affecting survival of the cell. By the same token, it is quite possible to increase expression of certain proteins to the point where they are toxic to the cell. Applicants' invention lies between these two extremes.

In general, there are two ways to improve antibody yield, theoretically. One is to increase total productivity of a given quantity of antibodies. There are limits on the improvements that can be made without affecting the individual antibody-expressing cells. While one can improve culture/fermentation conditions, improve spacing and the like, real world limitations on the cost and capability of processing hardware, the costs and frequency of media replacements, and the like combine to limit the improvements available by manipulating the environment in which the cells are grown to fractional or incremental improvements.

An alternative approach is to change the expression characteristics of the cells themselves. If substantial improvements in cell SPR can be made, without huge losses in volumetric productivity, and overall increase in antibody yield is obtained. Applicants have discovered that in fact SPR can be increased, as much as 300% or better, without a concomitant loss in productivity of a given volume of cells, giving an overall increase in antibody expression. Enhanced Antibody Production (EAP) is thus achieved by insertion of a DNA construct at a locus distant from the locus of the inserted antibody encoding sequence. This makes it possible to increase the level of expression without endangering the char-

5

acteristics of the antibody itself or the insert region, which may be critical to the expression of the heterologous antibody. Quality control is satisfied by ensuring that the mAb products of cells exhibiting EAP bind with the same relative avidity and affinity to the same target as cells of the parent strain, before enhancement.

The process is generally indicated in FIG. 1, which constitutes a kind of flow chart for the process of the invention. RHGP is used to inactivate one gene per cell in a population of cells, thus creating a RHGP library. The constituent cells of the library are subjected to a high throughput assay system for the detection of enhanced IgG production. The cells are altered using a Gene Search Vector (GSV) as illustrated in FIG. 2. When integrated into an allele of the target cell, the inserted construct is expressed—generating, in the embodiment illustrated, an anti-sense RNA which effectively reduces expression of the target protein. In alternative embodiments, the GSV may comprise a sequence or fragment which boosts expression of the target protein.

The constituent members of the transformed library are then subjected to a high throughput screening process, to identify candidates exhibiting EAP. One assay in particular that lends itself to this process is FACS. This is because transformed cells that express more antibody on their surface will secrete or release more antibodies. Thus, a rapid and high throughput low cost screening process selects out promising candidates whose mAb expression level are higher due to transformation by the GSV. To confirm that the high producers are in fact expressing the antibody of interest, the pool selected is subjected to a conventional ELISA assay, ensuring the antibodies secreted by the selected cells do in fact bind to the target antigen.

It will be appreciated that many cells will respond to the initial transformation by giving some gains in mAb SPR. To achieve the goals of this invention, that is enhancing SPR by as much as 1.5 fold, all the way up to 3-fold and beyond, only the most responsive transformants will be selected. FACS screening, as described above, permits rapid identification of EAP cells, in large amounts. This process is illustrated in FIG. 4, where a first selection of, e.g., the top 5% (the percentage collected will vary with the cell population, and it may be anything from 25% down to 5%—representative values being between those two endpoints, including 10, 15 and 20 percent by way of exemplification). This “first cut is expanded, and subjected to a second round of FACS sorting, again selecting a small percentage of the antibody-expressing cells showing the highest SPR. This second collection is then subjected to a third round, through single cell plating and culturing conditions—yielding stable populations of antibody-expressing cells exhibiting EAP and significantly higher SPRs than the original parent strain prior to manipulation through RHGP.

As shown by actual example discussed, *infra*, involving decreased expression of the Elmo1 gene, in fact, FACS can be used as described above, to enhance antibody-production values, and SPRs, of RHGP transformed cells. The repeated FACS selection “right-shifts” the population of antibodies, with each sorting giving rise to a population with a higher SPR—whether measured by mean, median or mode. The actual utility of FACS sorting according to the invention is illustrated in FIG. 5.

Total volume productivity (TVP) screens are faster and easier to do than selecting out individual improvements in SPR. Thus, the process can be accelerated by taking a total productivity measure for all the members of a transformed library. Since total productivity correlates with SPR, by selecting out high productivity lines, likely sources of high

6

mAb expressing cell lines are the highest volume productivity cell lines. Thus, FIG. 6 reflects an extinction experiment in which volume productivity for an entire library of potential transformants is measured, following RHGP. Thus, a number of cell lines actually show inferior volume productivity, while the majority show at least some degree of improvement, when compared with the non-transformed parent line.

The cell lines giving the highest volume productivity values from the experiment reflected in FIG. 6 (this was done with the Elmo1 experiment set forth below—giving actual experimental values) were measured for SPR as shown in FIG. 7. All but two of the cell lines giving a higher total productivity on a 9-day extinction experiment gave SPR values better than the parents—and as shown, the parents were selected for an already high SPR of 16 pg/cell/day. Cell lines expressing >50 pg/cell/day may be secured through this invention. This is illustrated in FIG. 8, where at least one cell line, 296C2H, prepared by RHGP insertion of the Elmo1 anti-sense RNA exhibited both SPR and volume productivity in excess of this target value. All of the selected cell lines illustrated show marked improvements in their SPR when compared to the high-producing parent. Thus, given a simple transformation step well away from the site of the transforming antibody sequences, significant increases in antibody expression are achieved. The correlation between SPR and total productivity is also shown in FIG. 9, which shows growth kinetics for the various cell lines. Depending on the envisaged facility and industrial or commercial process, growth kinetics may impact the choice of the “best” modified cell to select, given relatively similar TVP and SPR.

As noted above, it is important to develop a technique that is not only simple, susceptible of application on a rapid throughput format, and capable of giving substantial improvements in the SPR of a given mAb-producing cell line, it is essential that the transformation take place in a site remote from the antibody sequences themselves, so that antibody properties are not disturbed. As shown in FIG. 10, the antibodies of the RHGP transformed high SPR cells exhibit binding characteristics not distinguishable from those of the parent strain. In FIG. 10, the parent strain is given as the control. These increases are stable over time. See FIG. 11. Equally important is the transformation induced by RHGP pursuant to the invention results in stable increases in SPR. As shown in FIG. 12, a number of clones from a single experiment involving down regulation of the Elmo1 gene exhibited both higher SPR and higher TVP.

EXAMPLE 1

RHGP using Antisense RNA of the Elmo1 Gene

The Elmo1 gene of *C. elegans* was identified as important in phagocytosis of apoptotic cells, and for cell migration. Gumienny et al, Cell. 107(1): 27-41 (2001). This gene was targeted with an anti-sense knock-out RHGP, in an effort to improve higher antibody SPR in cells expressing recombinant antibodies. The general strategy described above was employed for this experiment.

Identification of Engulfment Cell Motility 1 Protein Gene Involved in Enhanced Antibody Production.

When the individual phenotypes have been selected for cloning, the target gene involved in enhanced antibody production was identified by the strategy shown in FIG. 1. The vector map for the Elmo1 construct is given in FIG. 14.

The full-length CHO ELMO1 cDNA was cloned into the expression vectors of pCDNA3.1 and pLlexp with both ori-

entations, which allow the over-expression of the ELMO1 protein or production of the antisense RNA. Since the anti-ITP antibody is not available, the CHO ITP cDNA was fused with myc tag at its 5' or 3' end and cloned into pLLexp expression vector. The fusion partner, myc tag will provide a domain for detection for the expressed ITP protein level.

To verify that the phenotypes with higher SPR have the GSV insertion in the genomes, the genomic DNA was first subjected to PCR amplification of the chloramphenicol acetyltransferase (CAT) gene. Indeed, the PCR analysis has indicated that all the single clones and the pools selected by FACS have the CAT gene inserted in the genome. To identify the gene involved in the phenotype of clone 296-C2H, the genomic DNA was digested with restriction enzymes individually, which allow us to rescue the genomic DNA along with the GSV. The digested genomic DNA was re-circulated and used to transform *E. coli* competent cells. A total of 16-24 transformed colonies were picked for DNA preparation and sequencing analysis with the LTR primers near the junctions between the GSV vector and the genomic DNA. The regenerated genomic sequence was taken for Blast Search in GeneBank. A 450-bp domain of CHO genomic DNA sequence shares 87% identities with the sequence on mouse chromosome 13, in which a gene called engulfment and cell motility1 protein (ELMO1) was located. Especially, the further sequencing information revealed that the corresponding exon 16 domain of CHO cell s shares 95% homology with mouse counterpart. Although the CHO genome sequence database is not available in public databases, it's obvious that the GSV has been integrated in the intron between the exon 15 and 16 in 296-C2H genome and interrupted the ELMO1 gene according to the blast search information. The CMV promoter from the GSV seems to transcribe the antisense RNA and knockdown the ELMO1 gene in the phenotype, which has lead to the antibody production enhancement. The ELMO1 gene has been identified from many other species, such as mouse, rat and human, which has been reported to be involved in the cells motility and required for cell phagocytosis and cells migration. A 3.7-kb full-length ELMO1 cDNA was isolated from a CHO cDNA library using a 31 nucleotide primer designed from exon 16 of CHO ELMO1. The complete coding sequence of ELMO1 from CHO cells is 2181-bp long encoding 727 amino acids protein. The CHO protein shares 99% homology with mouse, rat and human homolog. (FIG. 13). The cDNA was then cloned in pCDNA3.1 and pLLexp expression vector with both orientations for validation of the gene in naive cell line (FIG. 14)

As discussed above, downregulation of the Elmo1 gene, following insertion of the Elmo1 anti-sense "knockout" construct is correlated with high SPR in RGHP clones from this experiment. See FIG. 15. Importantly however, while some downregulation was observed, it was partial. Elmo-1 is still being produced, as would be expected, given the single allele insertion. In contrast, the increase in SPR and TVP was profound. The two correlated events, induced by a single round of RHGP followed by selection as described above, are shown in a single frame in FIG. 16.

EXAMPLE 2

Ion Transporter Protein

To demonstrate the efficacy of this invention, a second target for RHGP was selected, this time an ion transport protein. What is of fundamental importance is that this experiment demonstrates that proteins can be downregulated (underexpressed as compared with the parent strain expressing

the antibody of interest) or upregulated (overexpressed as compared with the unmodified parent strain expressing the antibody of interest) and nonetheless give EAP. What is fundamentally important is that the invention provides a method for modifying the expression pattern of at least one protein of a genome, coupled with a facile method for rapid detection and sequestration of cells expressing antibodies at a significantly higher SPR than the parent cell line prior to transformation by RHGP.

Identification of Ion Transporter Protein Gene Homolog Involved in Enhanced Antibody Production.

Using the same strategy, we have successfully identified the insertion site of the GSV in the genome of another clone 263-C4G. The genomic sequence contig was taken for Blast Search in GeneBank. The genomic DNA sequence of 263-C4G shares significantly high homology with that on mouse chromosome 13, in which the ion transporter protein gene homolog (ITP) was located 15 kb downstream of the GSV insertion site. Most likely, the CMV promoter of GSV has over-expressed the ITP homolog and lead to the enhancement of antibody production in the phenotype.

The cDNA of ITP gene was isolated by RT-PCR with mRNA of 263-C4G. The 2043-bp cDNA encodes 681 amino acids protein, which shares 96% identities with rat, and 95% with mouse and human homolog (FIG. 17). The ITP homolog belongs to the sugar-type transporter for the movement of substances such as ions, small molecules and micromolecules.

Methods—Preparation of RNA and Genomic DNA.

The total RNA was isolated from CHO cells using TRIzol Reagent (Invitrogen). Following the manufacturer's protocol, $5-10 \times 10^6$ CHO cells were used for each preparation. The mRNA was isolated using oligo dT magnetic beads (Invitrogen). To isolate the genomic DNA, the CHO cells ($5-10 \times 10^6$ cells) were collected and washed once with PBS solution. The cell pellet was resuspended in 10 ml of lysis buffer containing 0.32 M Sucrose, 10 mM Tris pH 7.5, 5 mM $MgCl_2$ and 1% Triton X-100. The cell lysate was centrifuged at $1500 \times g$ for 15 min. The supernatant was removed and the pellet was resuspended in 0.5 ml of proteinase K buffer containing 25 mM EDTA, 150 mM NaCl and 40 mM Tris pH 7.5 and transferred to a 1.5-ml tube. Immediately, 10 μ l of 10 mg/ml proteinase K stock solution and 25 μ l of 10% SDS were added to the mixture. The solution was mixed gently and incubated at 37° C. overnight. The next day, 5 μ l of 10 mg/ml of RNase A was added and incubated at 37° C. for 2-4 hrs. After RNase A digestion, the DNA mixture was extracted twice with phenol/isoamyl alcohol/chloroform. The DNA was then precipitated with equal volume of isopropanol and centrifuged at 14000 rpm for 15 min. The pellet was washed with 70% ethanol and dissolved in 200 μ l of TE (pH 7.5) buffer. The DNA concentration was determined by OD reading at A_{260} .

Genomic DNA Cloning.

To identify the genomic DNA sequence surrounding the GSV insertion site, 10 μ g of each genomic DNA in 250 μ l was digested with restriction enzyme, such as BamHI and HindIII. The digested DNA was then extracted once with phenol/isoamyl alcohol/chloroform and precipitated with 2.5 volumes of ethanol. The DNA was air dried and dissolved in 30 μ l of TE buffer. The digested DNA was then self-ligated with T4 ligase at 16° C. overnight. The next day, the ligated DNA was precipitated with ethanol and dissolved in 20 μ l of TE buffer. The ligated DNA was used for electroporation with ElectroMax DH10B competent cells. Sixteen colonies from

each ligated DNA were grown in 1.5 ml culture for DNA preparation and digestion with the restriction enzyme for size analysis. The plasmid DNA was further analyzed by DNA sequencing.

GenBank Blast Search and Genome Mapping.

The DNA sequences were taken for mouse genome homolog search through NCBI Blast Search program. When the mouse homolog has been identified at the insertion site, the genes in that locus surrounding the GSV could be scanned and identified. The orientation of the CMV promoter in GSV will decide either the gene has been knockdown or over-expressed by RHGP. If there was no homology identified, the DNA sequencing will be continued until the mouse homolog has been found.

Construction of the CHO cDNA Library.

The cDNA library was constructed with Invitrogen's SuperScript cDNA System. Following the manufacturer's protocol, the synthesized double stranded cDNA was ligated into a vector followed by transformation with ElectroMax DH10B competent cells. Two million transformants from the electroporation mixture were used to inoculate 100 ml of the TB broth medium at 37° C. for overnight. The plasmid DNA of the library was isolated with a Qiagen kit.

PCR Amplification of ITP cDNA.

Since the exon sequence of CHO ionic transporter protein is not available, the target cDNA was amplified by PCR with degenerate primers designed from the mouse ITP homolog. A 734-bp cDNA fragment in the middle of the gene was first amplified with a pair of degenerate primers (L625: 5'AACGTGGTCAGCAARTGGGA3' and R1339:

5'TTCACYTCRTGGCCCATCAT3'). The amplified cDNA fragment was completely sequenced. The 5' and 3' fragments of the gene were subsequently amplified with the primers designed from the known sequences of the internal fragment combined with the 5' and 3' primers designed from the mouse ITP homolog. After the 5' and 3' fragments of the gene were amplified and sequenced, the full-length ITP cDNA was finally amplified by PCR with the primers designed from both ends of the gene (ITP-L1: 5' CCCTGGCCATGGCGATA-GAY 3' and C4G-R3: 5' GGTCTGTAAACCTGTGTGCA 3').

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. Of particular note is the fact that the expression pattern of at least one gene of a genome of a cell line expressing an antibody of interest is altered, followed by rapid screening to identify elevated SPR. Identification of candidates offering EAP, in terms of both SPR and TVP leads to expansion and stabilization of those cell lines using standard procedure, as modified for each cell line type, and in light of the modification leading to underexpression or overexpression of the targeted gene. All such modifications are intended to be within the scope of the claims appended hereto.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 12

<210> SEQ ID NO 1

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: degenerate primer designed from the mouse ITP homolog

<400> SEQUENCE: 1

aacgtggtca gcaartggga

20

<210> SEQ ID NO 2

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: degenerate primer designed from the mouse ITP homolog

<400> SEQUENCE: 2

ttcacytcr t ggcccatcat

20

<210> SEQ ID NO 3

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: degenerate primer designed from the mouse ITP homolog

-continued

<400> SEQUENCE: 3

ccctggccat ggcgatagay

20

<210> SEQ ID NO 4

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: degenerate primer designed from the mouse ITP homolog

<400> SEQUENCE: 4

ggtctgtaaa cctgtgtgca

20

<210> SEQ ID NO 5

<211> LENGTH: 727

<212> TYPE: PRT

<213> ORGANISM: Homo sapien

<400> SEQUENCE: 5

Met Pro Pro Pro Ala Asp Ile Val Lys Val Ala Ile Glu Trp Pro Gly
1 5 10 15Ala Tyr Pro Lys Leu Met Glu Ile Asp Gln Lys Lys Pro Leu Ser Ala
20 25 30Ile Ile Lys Glu Val Cys Asp Gly Trp Ser Leu Ala Asn His Glu Tyr
35 40 45Phe Ala Leu Gln His Ala Asp Ser Ser Asn Phe Tyr Ile Thr Glu Lys
50 55 60Asn Arg Asn Glu Ile Lys Asn Gly Thr Ile Leu Arg Leu Thr Thr Ser
65 70 75 80Pro Ala Gln Asn Ala Gln Gln Leu His Glu Arg Ile Gln Ser Ser Ser
85 90 95Met Asp Ala Lys Leu Glu Ala Leu Lys Asp Leu Ala Ser Leu Ser Arg
100 105 110Asp Val Thr Phe Ala Gln Glu Phe Ile Asn Leu Asp Gly Ile Ser Leu
115 120 125Leu Thr Gln Met Val Glu Ser Gly Thr Glu Arg Tyr Gln Lys Leu Gln
130 135 140Lys Ile Met Lys Pro Cys Phe Gly Asp Met Leu Ser Phe Thr Leu Thr
145 150 155 160Ala Phe Val Glu Leu Met Asp His Gly Ile Val Ser Trp Asp Thr Phe
165 170 175Ser Val Ala Phe Ile Lys Lys Ile Ala Ser Phe Val Asn Lys Ser Ala
180 185 190Ile Asp Ile Ser Ile Leu Gln Arg Ser Leu Ala Ile Leu Glu Ser Met
195 200 205Val Leu Asn Ser His Asp Leu Tyr Gln Lys Val Ala Gln Glu Ile Thr
210 215 220Ile Gly Gln Leu Ile Pro His Leu Gln Gly Ser Asp Gln Glu Ile Gln
225 230 235 240Thr Tyr Thr Ile Ala Val Ile Asn Ala Leu Phe Leu Lys Ala Pro Asp
245 250 255Glu Arg Arg Gln Glu Met Ala Asn Ile Leu Ala Gln Lys Gln Leu Arg
260 265 270Ser Ile Ile Leu Thr His Val Ile Arg Ala Gln Arg Ala Ile Asn Asn
275 280 285

-continued

Glu Met Ala His Gln Leu Tyr Val Leu Gln Val Leu Thr Phe Asn Leu
 290 295 300

Leu Glu Asp Arg Met Met Thr Lys Met Asp Pro Gln Asp Gln Ala Gln
 305 310 315 320

Arg Asp Ile Ile Phe Glu Leu Arg Arg Ile Ala Phe Asp Ala Glu Ser
 325 330 335

Glu Pro Asn Asn Ser Ser Gly Ser Met Glu Lys Arg Lys Ser Met Tyr
 340 345 350

Thr Arg Asp Tyr Lys Lys Leu Gly Phe Ile Asn His Val Asn Pro Ala
 355 360 365

Met Asp Phe Thr Gln Thr Pro Pro Gly Met Leu Ala Leu Asp Asn Met
 370 375 380

Leu Tyr Phe Ala Lys His His Gln Asp Ala Tyr Ile Arg Ile Val Leu
 385 390 395 400

Glu Asn Ser Ser Arg Glu Asp Lys His Glu Cys Pro Phe Gly Arg Ser
 405 410 415

Ser Ile Glu Leu Thr Lys Met Leu Cys Glu Ile Leu Lys Val Gly Glu
 420 425 430

Leu Pro Ser Glu Thr Cys Asn Asp Phe His Pro Met Phe Phe Thr His
 435 440 445

Asp Arg Ser Phe Glu Glu Phe Phe Cys Ile Cys Ile Gln Leu Leu Asn
 450 455 460

Lys Thr Trp Lys Glu Met Arg Ala Thr Ser Glu Asp Phe Asn Lys Val
 465 470 475 480

Met Gln Val Val Lys Glu Gln Val Met Arg Ala Leu Thr Thr Lys Pro
 485 490 495

Ser Ser Leu Asp Gln Phe Lys Ser Lys Leu Gln Asn Leu Ser Tyr Thr
 500 505 510

Glu Ile Leu Lys Ile Arg Gln Ser Glu Arg Met Asn Gln Glu Asp Phe
 515 520 525

Gln Ser Arg Pro Ile Leu Glu Leu Lys Glu Lys Ile Gln Pro Glu Ile
 530 535 540

Leu Glu Leu Ile Lys Gln Gln Arg Leu Asn Arg Leu Val Glu Gly Thr
 545 550 555 560

Cys Phe Arg Lys Leu Asn Ala Arg Arg Arg Gln Asp Lys Phe Trp Tyr
 565 570 575

Cys Arg Leu Ser Pro Asn His Lys Val Leu His Tyr Gly Asp Leu Glu
 580 585 590

Glu Ser Pro Gln Gly Glu Val Pro His Asp Ser Leu Gln Asp Lys Leu
 595 600 605

Pro Val Ala Asp Ile Lys Ala Val Val Thr Gly Lys Asp Cys Pro His
 610 615 620

Met Lys Glu Lys Gly Ala Leu Lys Gln Asn Lys Glu Val Leu Glu Leu
 625 630 635 640

Ala Phe Ser Ile Leu Tyr Asp Ser Asn Cys Gln Leu Asn Phe Ile Ala
 645 650 655

Pro Asp Lys His Glu Tyr Cys Ile Trp Thr Asp Gly Leu Asn Ala Leu
 660 665 670

Leu Gly Lys Asp Met Met Ser Asp Leu Thr Arg Asn Asp Leu Asp Thr
 675 680 685

Leu Leu Ser Met Glu Ile Lys Leu Arg Leu Leu Asp Leu Glu Asn Ile
 690 695 700

Gln Ile Pro Asp Ala Pro Pro Pro Ile Pro Lys Glu Pro Ser Asn Tyr

-continued

705	710	715	720
Asp Phe Val Tyr Asp Cys Asn			
	725		
<p><210> SEQ ID NO 6 <211> LENGTH: 727 <212> TYPE: PRT <213> ORGANISM: Mouse</p> <p><400> SEQUENCE: 6</p>			
Met Pro Pro Pro Ser Asp Ile Val Lys Val Ala Ile Glu Trp Pro Gly			
1	5	10	15
Ala Tyr Pro Lys Leu Met Glu Ile Asp Gln Lys Lys Pro Leu Ser Ala			
	20	25	30
Ile Ile Lys Glu Val Cys Asp Gly Trp Ser Leu Ala Asn His Glu Tyr			
	35	40	45
Phe Ala Leu Gln His Ala Asp Ser Ser Asn Phe Tyr Ile Thr Glu Lys			
50	55	60	
Asn Arg Asn Glu Ile Lys Asn Gly Thr Ile Leu Arg Leu Thr Thr Ser			
65	70	75	80
Pro Ala Gln Asn Ala Gln Gln Leu His Glu Arg Ile Gln Ser Ser Ser			
	85	90	95
Met Asp Ala Lys Leu Glu Ala Leu Lys Asp Leu Ala Ser Leu Ser Arg			
	100	105	110
Asp Val Thr Phe Ala Gln Glu Phe Ile Asn Leu Asp Gly Ile Ser Leu			
	115	120	125
Leu Thr Gln Met Val Glu Ser Gly Thr Glu Arg Tyr Gln Lys Leu Gln			
	130	135	140
Lys Ile Met Lys Pro Cys Phe Gly Asp Met Leu Ser Phe Thr Leu Thr			
145	150	155	160
Ala Phe Val Glu Leu Met Asp His Gly Ile Val Ser Trp Asp Thr Phe			
	165	170	175
Ser Val Ala Phe Ile Lys Lys Ile Ala Ser Phe Val Asn Lys Ser Ala			
	180	185	190
Ile Asp Ile Ser Ile Leu Gln Arg Ser Leu Ala Ile Leu Glu Ser Met			
	195	200	205
Val Leu Asn Ser His Asp Leu Tyr Gln Lys Val Ala Gln Glu Ile Thr			
	210	215	220
Ile Gly Gln Leu Ile Pro His Leu Gln Gly Thr Asp Gln Glu Ile Gln			
225	230	235	240
Thr Tyr Thr Ile Ala Val Ile Asn Ala Leu Phe Leu Lys Ala Pro Asp			
	245	250	255
Glu Arg Arg Gln Glu Met Ala Asn Ile Leu Ala Gln Lys Gln Leu Arg			
	260	265	270
Tyr Ile Ile Leu Thr His Val Ile Arg Ala Gln Arg Ala Ile Asn Asn			
	275	280	285
Glu Met Ala His Gln Leu Tyr Val Leu Gln Val Leu Thr Phe Asn Leu			
	290	295	300
Leu Glu Asp Arg Met Met Thr Lys Met Asp Pro Gln Asp Gln Ala Gln			
305	310	315	320
Arg Asp Ile Ile Phe Glu Leu Arg Arg Ile Ala Phe Asp Ala Glu Ser			
	325	330	335
Glu Pro Asn Asn Ser Ser Gly Ser Met Glu Lys Arg Lys Ser Met Tyr			
	340	345	350

-continued

Thr Arg Asp Tyr Lys Lys Leu Gly Phe Ile Asn His Val Asn Pro Ala
 355 360 365

Met Asp Phe Thr Gln Thr Pro Pro Gly Met Leu Ala Leu Asp Asn Met
 370 375 380

Leu Tyr Phe Ala Lys His His Gln Asp Ala Tyr Ile Arg Ile Val Leu
 385 390 395 400

Glu Asn Ser Ser Arg Glu Asp Lys His Glu Cys Pro Phe Gly Arg Ser
 405 410 415

Ser Ile Glu Leu Thr Lys Met Leu Cys Glu Ile Leu Lys Val Gly Glu
 420 425 430

Leu Pro Ser Glu Thr Cys Asn Asp Phe His Pro Met Phe Phe Thr His
 435 440 445

Asp Arg Ser Phe Glu Glu Phe Phe Cys Ile Cys Ile Gln Leu Leu Asn
 450 455 460

Lys Thr Trp Lys Glu Met Arg Ala Thr Ser Glu Asp Phe Asn Lys Val
 465 470 475 480

Met Gln Val Val Lys Glu Gln Val Met Arg Ala Leu Thr Thr Lys Pro
 485 490 495

Ser Ser Leu Asp Gln Phe Lys Ser Lys Leu Gln Asn Leu Ser Tyr Thr
 500 505 510

Glu Ile Leu Lys Ile Arg Gln Ser Glu Arg Met Asn Gln Glu Asp Phe
 515 520 525

Gln Ser Arg Pro Ile Leu Glu Leu Lys Glu Lys Ile Gln Pro Glu Ile
 530 535 540

Leu Glu Leu Ile Lys Gln Gln Arg Leu Asn Arg Leu Val Glu Gly Thr
 545 550 555 560

Cys Phe Arg Lys Leu Asn Ala Arg Arg Arg Gln Asp Lys Phe Trp Tyr
 565 570 575

Cys Arg Leu Ser Pro Asn His Lys Val Leu His Tyr Gly Asp Leu Glu
 580 585 590

Glu Ser Pro Gln Gly Glu Val Pro His Asp Ser Leu Gln Asp Lys Leu
 595 600 605

Pro Val Ala Asp Ile Lys Ala Val Val Thr Gly Lys Asp Cys Pro His
 610 615 620

Met Lys Glu Lys Gly Ala Leu Lys Gln Asn Lys Glu Val Leu Glu Leu
 625 630 635 640

Ala Phe Ser Ile Leu Tyr Asp Ser Asn Cys Gln Leu Asn Phe Ile Ala
 645 650 655

Pro Asp Lys His Glu Tyr Cys Ile Trp Thr Asp Gly Leu Asn Ala Leu
 660 665 670

Leu Gly Lys Asp Met Met Ser Asp Leu Thr Arg Asn Asp Leu Asp Thr
 675 680 685

Leu Leu Ser Met Glu Ile Lys Leu Arg Leu Leu Asp Leu Glu Asn Ile
 690 695 700

Gln Ile Pro Asp Ala Pro Pro Pro Ile Pro Lys Glu Pro Ser Asn Tyr
 705 710 715 720

Asp Phe Val Tyr Asp Cys Asn
 725

<210> SEQ ID NO 7
 <211> LENGTH: 727
 <212> TYPE: PRT
 <213> ORGANISM: Rat

 <400> SEQUENCE: 7

-continued

Met Pro Pro Pro Ser Asp Ile Val Lys Val Ala Ile Glu Trp Pro Gly
 1 5 10 15
 Ala Tyr Pro Lys Leu Met Glu Ile Asp Gln Lys Lys Pro Leu Ser Ala
 20 25 30
 Ile Ile Lys Glu Val Cys Asp Gly Trp Ser Leu Ala Asn His Glu Tyr
 35 40 45
 Phe Ala Leu Gln His Ala Asp Ser Ser Asn Phe Tyr Ile Thr Glu Lys
 50 55 60
 Asn Arg Asn Glu Ile Lys Asn Gly Thr Ile Leu Arg Leu Thr Thr Ser
 65 70 75 80
 Pro Ala Gln Asn Ala Gln Gln Leu His Glu Arg Ile Gln Ser Ser Ser
 85 90 95
 Met Asp Ala Lys Leu Glu Ala Leu Lys Asp Leu Ala Ser Leu Ser Arg
 100 105 110
 Asp Val Thr Phe Ala Gln Glu Phe Ile Asn Leu Asp Gly Ile Ser Leu
 115 120 125
 Leu Thr Gln Met Val Glu Ser Gly Thr Glu Arg Tyr Gln Lys Leu Gln
 130 135 140
 Lys Ile Met Lys Pro Cys Phe Gly Asp Met Leu Ser Phe Thr Leu Thr
 145 150 155 160
 Ala Phe Val Glu Leu Met Asp His Gly Ile Val Ser Trp Asp Thr Phe
 165 170 175
 Ser Val Ala Phe Ile Lys Lys Ile Ala Ser Phe Val Asn Lys Ser Ala
 180 185 190
 Ile Asp Ile Ser Ile Leu Gln Arg Ser Leu Ala Ile Leu Glu Ser Met
 195 200 205
 Val Leu Asn Ser His Asp Leu Tyr Gln Lys Val Ala Gln Glu Ile Thr
 210 215 220
 Ile Gly Gln Leu Ile Pro His Leu Gln Gly Thr Asp Gln Glu Ile Gln
 225 230 235 240
 Thr Tyr Thr Ile Ala Val Ile Asn Ala Leu Phe Leu Lys Ala Pro Asp
 245 250 255
 Glu Arg Arg Gln Glu Met Ala Asn Ile Leu Ala Gln Lys Gln Leu Arg
 260 265 270
 Tyr Ile Ile Leu Thr His Val Ile Arg Ala Gln Arg Ala Ile Asn Asn
 275 280 285
 Glu Met Ala His Gln Leu Tyr Val Leu Gln Val Leu Thr Phe Asn Leu
 290 295 300
 Leu Glu Asp Arg Met Met Thr Lys Met Asp Pro Gln Asp Gln Ala Gln
 305 310 315 320
 Arg Asp Ile Ile Phe Glu Leu Arg Arg Ile Ala Phe Asp Ala Glu Ser
 325 330 335
 Glu Pro Asn Asn Ser Ser Gly Ser Met Glu Lys Arg Lys Ser Met Tyr
 340 345 350
 Thr Arg Asp Tyr Lys Lys Leu Gly Phe Ile Asn His Val Asn Pro Ala
 355 360 365
 Met Asp Phe Thr Gln Thr Pro Pro Gly Met Leu Ala Leu Asp Asn Met
 370 375 380
 Leu Tyr Phe Ala Lys His His Gln Asp Ala Tyr Ile Arg Ile Val Leu
 385 390 395 400
 Glu Asn Ser Ser Arg Glu Asp Lys His Glu Cys Pro Phe Gly Arg Ser
 405 410 415

-continued

Ser Ile Glu Leu Thr Lys Met Leu Cys Glu Ile Leu Lys Val Gly Glu
 420 425 430
 Leu Pro Ser Glu Thr Cys Asn Asp Phe His Pro Met Phe Phe Thr His
 435 440 445
 Asp Arg Ser Phe Glu Glu Phe Phe Cys Ile Cys Ile Gln Leu Leu Asn
 450 455 460
 Lys Thr Trp Lys Glu Met Arg Ala Thr Ser Glu Asp Phe Asn Lys Val
 465 470 475 480
 Met Gln Val Val Lys Glu Gln Val Met Arg Ala Leu Thr Thr Lys Pro
 485 490 495
 Ser Ser Leu Asp Gln Phe Lys Ser Lys Leu Gln Asn Leu Ser Tyr Thr
 500 505 510
 Glu Ile Leu Lys Ile Arg Gln Ser Glu Arg Met Asn Gln Glu Asp Phe
 515 520 525
 Gln Ser Arg Pro Ile Leu Glu Leu Lys Glu Lys Ile Gln Pro Glu Ile
 530 535 540
 Leu Glu Leu Ile Lys Gln Gln Arg Leu Asn Arg Leu Val Glu Gly Thr
 545 550 555 560
 Cys Phe Arg Lys Leu Asn Ala Arg Arg Arg Gln Asp Lys Phe Trp Tyr
 565 570 575
 Cys Arg Leu Ser Pro Asn His Lys Val Leu His Tyr Gly Asp Leu Glu
 580 585 590
 Glu Ser Pro Gln Gly Glu Val Pro His Asp Ser Leu Gln Asp Lys Leu
 595 600 605
 Pro Val Ala Asp Ile Lys Ala Val Val Thr Gly Lys Asp Cys Pro His
 610 615 620
 Met Lys Glu Lys Gly Ala Leu Lys Gln Asn Lys Glu Val Leu Glu Leu
 625 630 635 640
 Ala Phe Ser Ile Leu Tyr Asp Ser Asn Cys Gln Leu Asn Phe Ile Ala
 645 650 655
 Pro Asp Lys His Glu Tyr Cys Ile Trp Thr Asp Gly Leu Asn Ala Leu
 660 665 670
 Leu Gly Lys Asp Met Met Ser Asp Leu Thr Arg Asn Asp Leu Asp Thr
 675 680 685
 Leu Leu Ser Met Glu Ile Lys Leu Arg Leu Leu Asp Leu Glu Asn Ile
 690 695 700
 Gln Ile Pro Asp Ala Pro Pro Pro Ile Pro Lys Glu Pro Ser Asn Tyr
 705 710 715 720
 Asp Phe Val Tyr Asp Cys Asn
 725

<210> SEQ ID NO 8
 <211> LENGTH: 727
 <212> TYPE: PRT
 <213> ORGANISM: Chinese Hamster

<400> SEQUENCE: 8

Met Pro Pro Pro Ser Asp Ile Val Lys Val Ala Ile Glu Trp Pro Gly
 1 5 10 15
 Ala Tyr Pro Lys Leu Met Glu Ile Asp Gln Lys Lys Pro Leu Ser Ala
 20 25 30
 Ile Ile Lys Glu Val Cys Asp Gly Trp Ser Leu Ala Asn His Glu Tyr
 35 40 45
 Phe Ala Leu Gln His Ala Asp Ser Ser Asn Phe Tyr Ile Thr Glu Lys
 50 55 60

-continued

Asn	Arg	Asn	Glu	Ile	Lys	Asn	Gly	Thr	Ile	Leu	Arg	Leu	Thr	Thr	Ser
65					70					75					80
Pro	Ala	Gln	Asn	Ala	Gln	Gln	Leu	His	Glu	Arg	Ile	Gln	Ser	Ser	Ser
				85					90					95	
Met	Asp	Ala	Lys	Leu	Glu	Ala	Leu	Lys	Asp	Leu	Ala	Ser	Leu	Ser	Arg
			100					105					110		
Asp	Val	Thr	Phe	Ala	Gln	Glu	Phe	Ile	Asn	Leu	Asp	Gly	Ile	Ser	Leu
		115						120				125			
Leu	Thr	Gln	Met	Val	Glu	Ser	Gly	Thr	Glu	Arg	Tyr	Gln	Lys	Leu	Gln
	130					135					140				
Lys	Ile	Met	Lys	Pro	Cys	Phe	Gly	Asp	Met	Leu	Ser	Phe	Thr	Leu	Thr
145					150					155					160
Ala	Phe	Val	Glu	Leu	Met	Asp	His	Gly	Ile	Val	Ser	Trp	Asp	Thr	Phe
				165					170						175
Ser	Val	Ala	Phe	Ile	Lys	Lys	Ile	Ala	Ser	Phe	Val	Asn	Lys	Ser	Ala
			180					185					190		
Ile	Asp	Ile	Ser	Ile	Leu	Gln	Arg	Ser	Leu	Ala	Ile	Leu	Glu	Ser	Met
		195					200						205		
Val	Leu	Asn	Ser	His	Asp	Leu	Tyr	Gln	Lys	Val	Ala	Gln	Glu	Ile	Thr
	210					215					220				
Ile	Gly	Gln	Leu	Ile	Pro	His	Leu	Gln	Gly	Thr	Asp	Gln	Glu	Ile	Gln
225					230					235					240
Thr	Tyr	Thr	Ile	Ala	Val	Ile	Asn	Ala	Leu	Phe	Leu	Lys	Ala	Pro	Asp
				245					250					255	
Glu	Arg	Arg	Gln	Glu	Met	Ala	Asn	Ile	Leu	Ala	Gln	Lys	Gln	Leu	Arg
			260					265					270		
Tyr	Ile	Ile	Leu	Thr	His	Val	Ile	Arg	Ala	Gln	Arg	Ala	Ile	Asn	Asn
		275					280					285			
Glu	Met	Ala	His	Gln	Leu	Tyr	Val	Leu	Gln	Val	Leu	Thr	Phe	Asn	Leu
	290					295					300				
Leu	Glu	Asp	Arg	Met	Met	Thr	Lys	Met	Asp	Pro	Gln	Asp	Gln	Ala	Gln
305					310					315					320
Arg	Asp	Ile	Ile	Phe	Glu	Leu	Arg	Arg	Ile	Ala	Phe	Asp	Ala	Glu	Leu
				325					330					335	
Glu	Pro	Asn	Asn	Ser	Ser	Gly	Ser	Met	Glu	Lys	Arg	Lys	Ser	Met	Tyr
			340					345					350		
Thr	Arg	Asp	Tyr	Lys	Lys	Leu	Gly	Phe	Ile	Asn	His	Val	Asn	Pro	Ala
		355					360					365			
Met	Asp	Phe	Thr	Gln	Thr	Pro	Pro	Gly	Met	Leu	Ala	Leu	Asp	Asn	Met
	370					375					380				
Leu	Tyr	Phe	Ala	Lys	His	His	Gln	Asp	Ala	Tyr	Ile	Arg	Ile	Val	Leu
385					390					395					400
Glu	Asn	Ser	Ser	Arg	Glu	Asp	Lys	His	Glu	Cys	Pro	Phe	Gly	Arg	Ser
				405					410					415	
Ser	Ile	Glu	Leu	Thr	Lys	Met	Leu	Cys	Glu	Ile	Leu	Lys	Val	Gly	Glu
		420						425					430		
Leu	Pro	Ser	Glu	Thr	Cys	Asn	Asp	Phe	His	Pro	Met	Phe	Phe	Thr	His
		435					440					445			
Asp	Arg	Ser	Phe	Glu	Glu	Phe	Phe	Cys	Ile	Cys	Ile	Gln	Leu	Leu	Asn
	450					455					460				
Lys	Thr	Trp	Lys	Glu	Met	Arg	Ala	Thr	Ser	Glu	Asp	Phe	Asn	Lys	Val
465					470					475					480

-continued

Met Gln Val Val Lys Glu Gln Val Met Arg Ala Leu Thr Thr Lys Pro
485 490 495

Ser Ser Leu Asp Gln Phe Lys Ser Lys Leu Gln Asn Leu Ser Tyr Thr
500 505 510

Glu Ile Leu Lys Ile Arg Gln Ser Glu Arg Met Asn Gln Glu Asp Phe
515 520 525

Gln Ser Arg Pro Ile Leu Glu Leu Lys Glu Lys Ile Gln Pro Glu Ile
530 535 540

Leu Glu Leu Ile Lys Gln Gln Arg Leu Asn Arg Leu Val Glu Gly Thr
545 550 555 560

Cys Phe Arg Lys Leu Asn Ala Arg Arg Arg Gln Asp Lys Phe Trp Tyr
565 570 575

Cys Arg Leu Ser Pro Asn His Lys Val Leu His Tyr Gly Asp Leu Glu
580 585 590

Glu Ser Pro Gln Gly Glu Val Pro His Asp Ser Leu Gln Asp Lys Leu
595 600 605

Pro Val Ala Asp Ile Lys Ala Val Val Thr Gly Lys Asp Cys Pro His
610 615 620

Met Lys Glu Lys Gly Ala Leu Lys Gln Asn Lys Glu Val Leu Glu Leu
625 630 635 640

Ala Phe Ser Ile Leu Tyr Asp Ser Asn Cys Gln Leu Asn Phe Ile Ala
645 650 655

Pro Asp Lys His Glu Tyr Cys Ile Trp Thr Asp Gly Leu Asn Ala Leu
660 665 670

Leu Gly Lys Asp Met Leu Ser Asp Leu Thr Arg Asn Asp Leu Asp Thr
675 680 685

Leu Leu Ser Met Glu Ile Lys Leu Arg Leu Leu Asp Leu Glu Asn Ile
690 695 700

Gln Ile Pro Asp Ala Pro Pro Pro Ile Pro Lys Glu Pro Ser Asn Tyr
705 710 715 720

Asp Phe Val Tyr Asp Cys Asn
725

<210> SEQ ID NO 9
<211> LENGTH: 905
<212> TYPE: PRT
<213> ORGANISM: Rat

<400> SEQUENCE: 9

Met Ala Phe Gly Asn Leu Ala Cys Ile Val Cys Lys Leu His Phe Ile
1 5 10 15

Ser Lys Thr Asn Asp Asn Val Leu Ser Gly Cys Gly Leu Leu Gly Asp
20 25 30

Pro Cys Pro Ala Gln Arg Pro Pro Gly Ile Leu Arg Arg Pro Gly Cys
35 40 45

Pro Ser Pro Arg Pro Cys Val Cys Pro Gly Gly Gly Asp Gly Ala Arg
50 55 60

Leu Glu Gly Ala Leu Val Arg Arg Ser Gly Ala Ala Gly Trp Leu Arg
65 70 75 80

Ala Arg Gly Phe Arg Glu Arg Pro Lys Ser Val Glu Asp Leu Leu Thr
85 90 95

Arg Gly Arg Pro Arg Leu Gln His Asn Ala His Arg Cys Pro Gln Gly
100 105 110

Pro Glu Ser Arg Gln Gly Arg Gly Ser Gly Ser Ser Ser Ser Ala Ala
115 120 125

-continued

Ser Ala Gln Gln Arg Arg Ala Ser Glu Leu Gly Gly Arg Pro Gly Arg
 130 135 140

Gly Ala Asp Glu Pro Arg Arg Leu Arg Ala Arg Thr Ser Ser Ala Arg
 145 150 155 160

Arg Pro Gly Arg Gly Gly Ala Arg Val Thr Trp Gly Trp Pro Asp Ser
 165 170 175

Pro Gly Pro Glu His Ser Leu Arg Pro Arg Ala Leu Leu Leu Gly Gly
 180 185 190

Gly Ala Val Pro Gly Arg Pro Pro Ser Ser Ala Ala Leu Tyr Ala Pro
 195 200 205

Leu Arg Ala Pro Arg Ala Leu Ala Met Ala Ile Asp Arg Arg Arg Glu
 210 215 220

Ala Ala Gly Ser Gly Ala Gly Arg Gln Pro Ala Pro Ala Glu Glu Asn
 225 230 235 240

Gly Ser Leu Pro Pro Gly Asp Ala Ala Ala Ser Ala Pro Leu Gly Gly
 245 250 255

Arg Ala Gly Ser Gly Gly Ser Ala Glu Ile Gln Pro Leu Pro Ala Leu
 260 265 270

His Pro Ser Gly Gly Pro His Ser Ser Cys Cys Ala Ala Ala Ala Ala
 275 280 285

Pro Ser Leu Leu Leu Leu Asp Tyr Asp Gly Ser Val Leu Pro Phe Leu
 290 295 300

Gly Gly Leu Gly Gly Gly Tyr Gln Lys Thr Leu Val Val Leu Thr Trp
 305 310 315 320

Ile Pro Ala Leu Phe Ile Gly Phe Ser Gln Phe Ser Asp Ser Phe Leu
 325 330 335

Leu Asp Gln Pro Asn Phe Trp Cys His Gly Ala Gly Lys Gly Thr Glu
 340 345 350

Leu Ala Gly Ala Thr Val Thr Gly Arg Trp Gly Asp Met Gly Asn Trp
 355 360 365

Thr Ser Pro Ser Ala Thr Pro Phe Ser Thr Ala Ser Trp Gly Thr Thr
 370 375 380

Ser Asn Arg Ser Asn Ser Ser Asp Thr Pro Pro Leu Pro Ser Pro Pro
 385 390 395 400

Gly Lys Gly Asn Asn Asp Ser Asn Cys Glu Cys His Ala Trp Asp Tyr
 405 410 415

Gly Ile Arg Thr Gly Leu Val Gln Asn Val Val Ser Lys Trp Asp Leu
 420 425 430

Val Cys Asp Asn Ala Trp Lys Val His Val Ala Lys Phe Ser Leu Leu
 435 440 445

Val Gly Leu Ile Phe Gly Tyr Leu Ile Thr Gly Cys Ile Ala Asp Trp
 450 455 460

Val Gly Arg Arg Pro Val Leu Leu Phe Ser Val Ile Phe Ile Leu Ile
 465 470 475 480

Phe Gly Leu Thr Val Ala Leu Ser Val Asn Val Thr Met Phe Ser Thr
 485 490 495

Leu Arg Phe Phe Glu Gly Phe Cys Leu Ala Gly Ile Ile Leu Thr Leu
 500 505 510

Tyr Ala Leu Arg Ile Glu Leu Cys Pro Pro Gly Lys Arg Phe Ile Ile
 515 520 525

Thr Met Val Ala Ser Phe Val Ala Met Ala Gly Gln Phe Leu Met Pro
 530 535 540

-continued

Gly Leu Ala Ala Leu Cys Arg Asp Trp Gln Val Leu Gln Ala Leu Ile
 545 550 555 560
 Ile Cys Pro Phe Leu Leu Met Leu Leu Tyr Trp Ser Ile Phe Pro Glu
 565 570 575
 Ser Leu Arg Trp Leu Met Ala Thr Gln Gln Phe Glu Ser Ala Lys Lys
 580 585 590
 Leu Ile Leu Tyr Leu Thr Gln Lys Asn Cys Val Ser Pro Glu Ser Asp
 595 600 605
 Ile Lys Gly Val Met Pro Glu Leu Glu Lys Glu Leu Ser Arg Arg Pro
 610 615 620
 Lys Lys Val Cys Ile Val Lys Val Val Gly Thr Arg Asn Leu Trp Lys
 625 630 635 640
 Asn Ile Val Val Leu Cys Val Asn Ser Leu Thr Gly Tyr Gly Ile His
 645 650 655
 His Cys Phe Ala Arg Ser Met Met Gly His Glu Val Lys Val Pro Leu
 660 665 670
 Leu Glu Asn Phe Tyr Ala Asp Tyr Tyr Thr Thr Ala Ser Ile Ala Leu
 675 680 685
 Val Ser Cys Leu Ala Met Cys Val Val Val Arg Phe Leu Gly Arg Arg
 690 695 700
 Gly Gly Leu Leu Leu Phe Met Ile Leu Thr Ala Leu Ala Ser Leu Leu
 705 710 715 720
 Gln Leu Gly Leu Leu Asn Leu Ile Gly Lys Tyr Ser Gln His Pro Asp
 725 730 735
 Ser Glu Leu Gln Leu Lys Leu Ala Val Gly Met Ser Asp Ser Val Lys
 740 745 750
 Asp Lys Phe Ser Ile Ala Phe Ser Ile Val Gly Met Phe Ala Ser His
 755 760 765
 Ala Val Gly Ser Leu Ser Val Phe Phe Cys Ala Glu Ile Thr Pro Thr
 770 775 780
 Val Ile Arg Cys Gly Gly Leu Gly Leu Val Leu Ala Ser Ala Gln Phe
 785 790 795 800
 Gly Met Leu Thr Ala Pro Ile Ile Glu Leu His Asn Gln Lys Gly Tyr
 805 810 815
 Phe Leu His His Ile Ile Phe Ala Cys Cys Thr Leu Ile Cys Ile Ile
 820 825 830
 Cys Ile Leu Leu Leu Pro Glu Ser Arg Asp Gln Asn Leu Pro Glu Asn
 835 840 845
 Ile Ala Asn Gly Glu His Tyr Thr Arg Gln Pro Leu Leu Ser His Lys
 850 855 860
 Lys Gly Glu Gln Pro Leu Leu Leu Thr Asn Ala Glu Leu Lys Asp Tyr
 865 870 875 880
 Ser Gly Leu His Asp Val Ala Ala Val Gly Asp Gly Leu Ser Glu Gly
 885 890 895
 Ala Thr Ala Asn Gly Met Lys Thr Met
 900 905

<210> SEQ ID NO 10
 <211> LENGTH: 689
 <212> TYPE: PRT
 <213> ORGANISM: Mouse

<400> SEQUENCE: 10

Met Ala Ile Asp Arg Arg Arg Glu Ala Ala Gly Ser Gly Ala Gly Arg
 1 5 10 15

-continued

Gln Pro Ala Pro Ala Glu Glu Asn Gly Ser Leu Pro Pro Gly Asp Ala
 20 25 30
 Ala Ala Ser Ala Pro Leu Gly Gly Arg Ala Gly Ser Gly Gly Ser Ala
 35 40 45
 Glu Ile Gln Pro Leu Pro Ala Leu His Pro Ser Gly Gly Pro His Ser
 50 55 60
 Ser Cys Cys Ala Ala Ala Ala Ala Pro Ser Leu Leu Leu Leu Asp Tyr
 65 70 75 80
 Asp Gly Ser Val Leu Pro Phe Leu Gly Gly Leu Gly Gly Gly Tyr Gln
 85 90 95
 Lys Thr Leu Val Val Leu Thr Trp Ile Pro Ala Leu Phe Ile Gly Phe
 100 105 110
 Ser Gln Phe Ser Asp Ser Phe Leu Leu Asp Gln Pro Asn Phe Trp Cys
 115 120 125
 His Gly Ala Gly Lys Gly Thr Glu Leu Ala Gly Ala Thr Val Thr Gly
 130 135 140
 Arg Trp Gly Asp Met Gly Asn Trp Thr Ser Pro Ser Ala Thr Pro Phe
 145 150 155 160
 Ser Thr Ala Ser Trp Gly Thr Thr Ser Asn Arg Ser Asn Ser Ser Asp
 165 170 175
 Thr Pro Pro Leu Pro Ser Pro Pro Gly Lys Gly Asn Asn Asp Ser Asn
 180 185 190
 Cys Glu Cys His Ala Trp Asp Tyr Gly Ile Arg Thr Gly Leu Val Gln
 195 200 205
 Asn Val Val Ser Lys Trp Asp Leu Val Cys Asp Asn Ala Trp Lys Val
 210 215 220
 His Val Ala Lys Phe Ser Leu Leu Val Gly Leu Ile Phe Gly Tyr Leu
 225 230 235 240
 Ile Thr Gly Cys Ile Ala Asp Trp Val Gly Arg Arg Pro Val Leu Leu
 245 250 255
 Phe Ser Val Ile Phe Ile Leu Ile Phe Gly Leu Thr Val Ala Leu Ser
 260 265 270
 Val Asn Val Thr Met Phe Ser Thr Leu Arg Phe Phe Glu Gly Phe Cys
 275 280 285
 Leu Ala Gly Ile Ile Leu Thr Leu Tyr Ala Leu Arg Ile Glu Leu Cys
 290 295 300
 Pro Pro Gly Lys Arg Phe Met Ile Thr Met Val Ala Ser Phe Val Ala
 305 310 315 320
 Met Ala Gly Gln Phe Leu Met Pro Gly Leu Ala Ala Leu Cys Arg Asp
 325 330 335
 Trp Gln Val Leu Gln Ala Leu Ile Ile Cys Pro Phe Leu Leu Met Leu
 340 345 350
 Leu Tyr Trp Ser Ile Phe Pro Glu Ser Leu Arg Trp Leu Met Ala Thr
 355 360 365
 Gln Gln Phe Glu Ser Ala Lys Lys Leu Ile Leu Tyr Leu Thr Gln Lys
 370 375 380
 Asn Cys Val Ser Pro Glu Ser Asp Ile Lys Gly Val Met Pro Glu Leu
 385 390 395 400
 Glu Lys Glu Leu Ser Arg Arg Pro Lys Lys Val Cys Ile Val Lys Val
 405 410 415
 Val Gly Thr Arg Asn Leu Trp Lys Asn Ile Val Val Leu Cys Val Asn
 420 425 430

-continued

Ser Leu Thr Gly Tyr Gly Ile His His Cys Phe Ala Arg Ser Met Met
 435 440 445

Gly His Glu Val Lys Val Pro Leu Leu Glu Asn Phe Tyr Ala Asp Tyr
 450 455 460

Tyr Thr Thr Ala Ser Ile Ala Leu Val Ser Cys Leu Ala Met Cys Val
 465 470 475 480

Val Val Arg Phe Leu Gly Arg Arg Gly Gly Leu Leu Leu Phe Met Ile
 485 490 495

Leu Thr Ala Leu Ala Ser Leu Leu Gln Leu Gly Leu Leu Asn Leu Ile
 500 505 510

Gly Lys Tyr Ser Gln His Pro Asp Ser Glu Leu Gln Leu Lys Leu Ala
 515 520 525

Val Gly Met Ser Asp Ser Val Lys Asp Lys Phe Ser Ile Ala Phe Ser
 530 535 540

Ile Val Gly Met Phe Ala Ser His Ala Val Gly Ser Leu Ser Val Phe
 545 550 555 560

Phe Cys Ala Glu Ile Thr Pro Thr Val Ile Arg Cys Gly Gly Leu Gly
 565 570 575

Leu Val Leu Ala Ser Ala Gln Phe Gly Met Leu Thr Ala Pro Ile Ile
 580 585 590

Glu Leu His Asn Gln Lys Gly Tyr Phe Leu His His Ile Ile Phe Ala
 595 600 605

Cys Cys Thr Leu Ile Cys Ile Ile Cys Ile Leu Leu Leu Pro Glu Ser
 610 615 620

Arg Asp Gln Asn Leu Pro Glu Asn Ile Ala Asn Gly Glu His Tyr Thr
 625 630 635 640

Arg Gln Pro Leu Leu Ser His Lys Lys Gly Glu Gln Pro Leu Leu Leu
 645 650 655

Thr Asn Ala Glu Leu Lys Asp Tyr Ser Gly Leu His Asp Val Ala Ala
 660 665 670

Val Gly Asp Gly Leu Ser Glu Gly Ala Thr Ala Asn Gly Met Lys Ser
 675 680 685

Met

<210> SEQ ID NO 11

<211> LENGTH: 681

<212> TYPE: PRT

<213> ORGANISM: Chinese Hamster

<400> SEQUENCE: 11

Met Ala Ile Asp Arg Arg Arg Glu Ala Ala Gly Ser Gly Ala Gly Arg
 1 5 10 15

Gln Pro Ala Pro Ala Glu Glu Asn Gly Ser Leu Pro Pro Gly Asp Ala
 20 25 30

Ala Ala Ser Ala Pro Leu Gly Gly Arg Ala Gly Ser Gly Gly Ser Ala
 35 40 45

Glu Ile Gln Pro Leu Pro Ala Leu His Pro Ser Gly Gly Pro His Ser
 50 55 60

Ser Cys Cys Ala Ala Ala Ala Ala Pro Ser Leu Leu Leu Leu Asp Tyr
 65 70 75 80

Asp Gly Ser Val Leu Pro Phe Leu Gly Gly Leu Gly Gly Gly Tyr Gln
 85 90 95

Lys Thr Leu Val Val Leu Thr Trp Ile Pro Ala Leu Phe Ile Gly Phe
 100 105 110

-continued

Ser	Gln	Phe	Ser	Asp	Ser	Phe	Leu	Leu	Asp	Gln	Pro	Asn	Phe	Trp	Cys
		115					120					125			
His	Gly	Ala	Gly	Lys	Gly	Thr	Glu	Leu	Ala	Gly	Ala	Thr	Val	Thr	Gly
	130					135					140				
Arg	Trp	Gly	Asp	Met	Gly	Asn	Trp	Thr	Ser	Pro	Ser	Ala	Thr	Pro	Phe
145					150					155					160
Ser	Thr	Ala	Ser	Trp	Gly	Thr	Thr	Ser	Asn	Arg	Ser	Asn	Ser	Ser	Asp
				165					170					175	
Thr	Pro	Pro	Leu	Pro	Ser	Pro	Pro	Gly	Lys	Gly	Asn	Asn	Asp	Ser	Asn
			180					185					190		
Cys	Glu	Cys	His	Ala	Trp	Asp	Tyr	Gly	Ile	Arg	Thr	Gly	Leu	Val	Gln
		195					200					205			
Asn	Val	Val	Ser	Lys	Trp	Asp	Leu	Val	Cys	Asp	Asn	Ala	Trp	Lys	Val
	210					215					220				
His	Val	Ala	Lys	Phe	Ser	Leu	Leu	Val	Gly	Leu	Ile	Phe	Gly	Tyr	Leu
225					230					235					240
Ile	Thr	Gly	Cys	Ile	Ala	Asp	Trp	Val	Gly	Arg	Arg	Pro	Val	Leu	Leu
				245					250					255	
Phe	Ser	Val	Ile	Phe	Ile	Leu	Ile	Phe	Gly	Leu	Thr	Val	Ala	Leu	Ser
			260					265					270		
Val	Asn	Val	Thr	Met	Phe	Ser	Thr	Leu	Arg	Phe	Phe	Glu	Gly	Phe	Cys
		275					280					285			
Leu	Ala	Gly	Ile	Ile	Leu	Thr	Leu	Tyr	Ala	Leu	Arg	Ile	Glu	Leu	Cys
	290					295					300				
Pro	Pro	Gly	Lys	Arg	Phe	Met	Ile	Thr	Met	Val	Ala	Ser	Phe	Val	Ala
305					310					315					320
Met	Ala	Gly	Gln	Phe	Leu	Met	Pro	Gly	Leu	Ala	Ala	Leu	Cys	Arg	Asp
				325					330					335	
Trp	Gln	Val	Leu	Gln	Ala	Leu	Ile	Ile	Cys	Pro	Phe	Leu	Leu	Met	Leu
			340					345					350		
Leu	Tyr	Trp	Ser	Ile	Phe	Pro	Glu	Ser	Leu	Arg	Trp	Leu	Met	Ala	Thr
		355					360					365			
Gln	Gln	Phe	Glu	Ser	Ala	Lys	Lys	Leu	Ile	Leu	Tyr	Leu	Thr	Gln	Lys
		370				375					380				
Asn	Cys	Val	Ser	Pro	Glu	Ser	Asp	Ile	Lys	Gly	Val	Met	Pro	Glu	Leu
385					390					395					400
Glu	Lys	Glu	Leu	Ser	Arg	Arg	Pro	Lys	Lys	Val	Cys	Ile	Val	Lys	Val
				405					410					415	
Val	Gly	Thr	Arg	Asn	Leu	Trp	Lys	Asn	Ile	Val	Val	Leu	Cys	Val	Asn
			420					425					430		
Ser	Leu	Thr	Gly	Tyr	Gly	Ile	His	His	Cys	Phe	Ala	Arg	Ser	Met	Met
		435					440					445			
Gly	His	Glu	Val	Lys	Val	Pro	Leu	Leu	Glu	Asn	Phe	Tyr	Ala	Asp	Tyr
	450					455					460				
Tyr	Thr	Thr	Ala	Ser	Ile	Ala	Leu	Val	Ser	Cys	Leu	Ala	Met	Cys	Val
465					470					475					480
Val	Val	Arg	Phe	Leu	Gly	Arg	Arg	Gly	Gly	Leu	Leu	Leu	Phe	Met	Ile
				485					490					495	
Leu	Thr	Ala	Leu	Ala	Ser	Leu	Leu	Gln	Leu	Gly	Leu	Leu	Asn	Leu	Ile
			500					505					510		
Gly	Lys	Tyr	Ser	Gln	His	Pro	Asp	Ser	Gly	Met	Ser	Asp	Ser	Val	Lys
		515					520					525			
Asp	Lys	Phe	Ser	Ile	Ala	Phe	Ser	Ile	Val	Gly	Met	Phe	Ala	Ser	His

-continued

530	535	540
Ala Val Gly Ser Leu Ser Val Phe Phe Cys Ala Glu Ile Thr Pro Thr 545	550	555 560
Val Ile Arg Cys Gly Gly Leu Gly Leu Val Leu Ala Ser Ala Gln Phe 565	570	575
Gly Met Leu Thr Ala Pro Ile Ile Glu Leu His Asn Gln Lys Gly Tyr 580	585	590
Phe Leu His His Ile Ile Phe Ala Cys Cys Thr Leu Ile Cys Ile Ile 595	600	605
Cys Ile Leu Leu Leu Pro Glu Ser Arg Asp Gln Asn Leu Pro Glu Asn 610	615	620
Ile Ala Asn Gly Glu His Tyr Thr Arg Gln Pro Leu Leu Ser His Lys 625	630	635 640
Lys Gly Glu Gln Pro Leu Leu Leu Thr Asn Ala Glu Leu Lys Asp Tyr 645	650	655
Ser Gly Leu His Asp Val Ala Ala Val Gly Asp Gly Leu Ser Glu Gly 660	665	670
Ala Thr Ala Asn Gly Met Lys Ser Met 675	680	

<210> SEQ ID NO 12

<211> LENGTH: 371

<212> TYPE: PRT

<213> ORGANISM: Homo sapien

<400> SEQUENCE: 12

Met Ile Thr Met Val Ala Ser Phe Val Ala Met Ala Gly Gln Phe Leu 1	5	10	15
Met Pro Gly Leu Ala Ala Leu Cys Arg Asp Trp Gln Val Leu Gln Ala 20	25	30	
Leu Ile Ile Cys Pro Phe Leu Leu Met Leu Leu Tyr Trp Ser Ile Phe 35	40	45	
Pro Glu Ser Leu Arg Trp Leu Met Ala Thr Gln Gln Phe Glu Ser Ala 50	55	60	
Lys Lys Leu Ile Leu Tyr Leu Thr Gln Lys Asn Cys Val Ser Pro Glu 65	70	75	80
Ser Asp Ile Lys Gly Val Met Pro Glu Leu Glu Lys Glu Leu Ser Arg 85	90	95	
Arg Pro Lys Lys Val Cys Ile Val Lys Val Val Gly Thr Arg Asn Leu 100	105	110	
Trp Lys Asn Ile Val Val Leu Cys Val Asn Ser Leu Thr Gly Tyr Gly 115	120	125	
Ile His His Cys Phe Ala Arg Ser Met Met Gly His Glu Val Lys Val 130	135	140	
Pro Leu Leu Glu Asn Phe Tyr Ala Asp Tyr Tyr Thr Thr Ala Ser Ile 145	150	155	160
Ala Leu Val Ser Cys Leu Ala Met Cys Val Val Val Arg Phe Leu Gly 165	170	175	
Arg Arg Gly Gly Leu Leu Leu Phe Met Ile Leu Thr Ala Leu Ala Ser 180	185	190	
Leu Leu Gln Leu Gly Leu Leu Asn Leu Ile Gly Lys Tyr Ser Gln His 195	200	205	
Pro Asp Ser Gly Met Ser Asp Ser Val Lys Asp Lys Phe Ser Ile Ala 210	215	220	

-continued

Phe	Ser	Ile	Val	Gly	Met	Phe	Ala	Ser	His	Ala	Val	Gly	Ser	Leu	Ser
225					230					235					240
Val	Phe	Phe	Cys	Ala	Glu	Ile	Thr	Pro	Thr	Val	Ile	Arg	Cys	Gly	Gly
			245						250					255	
Leu	Gly	Leu	Val	Leu	Ala	Ser	Ala	Gln	Phe	Gly	Met	Leu	Thr	Ala	Pro
			260					265					270		
Ile	Ile	Glu	Leu	His	Asn	Gln	Lys	Gly	Tyr	Phe	Leu	His	His	Ile	Ile
		275					280					285			
Phe	Ala	Cys	Cys	Thr	Leu	Ile	Cys	Ile	Ile	Cys	Ile	Leu	Leu	Leu	Pro
	290						295				300				
Glu	Ser	Arg	Asp	Gln	Asn	Leu	Pro	Glu	Asn	Ile	Ala	Asn	Gly	Glu	His
305					310					315					320
Tyr	Thr	Arg	Gln	Pro	Leu	Leu	Ser	His	Lys	Lys	Gly	Glu	Gln	Pro	Leu
				325					330					335	
Leu	Leu	Thr	Asn	Ala	Glu	Leu	Lys	Asp	Tyr	Ser	Gly	Leu	His	Asp	Val
			340					345					350		
Ala	Ala	Val	Gly	Asp	Gly	Leu	Ser	Glu	Gly	Ala	Thr	Ala	Asn	Gly	Met
		355					360					365			
Lys	Gly	Met													
		370													

What is claimed is:

1. A method of enhancing the specific productivity ratio of antibody expression of a mammalian cell line which expresses an antibody of interest, comprising:

altering the expression pattern of at least one gene of the genome of said cell line other than said antibody through random homozygous gene perturbation (RHGP) to either increase or decrease the level of expression of said gene,

screening cells of said cell line transformed by RHGP to identify those transformed cells that exhibit a higher specific productivity rate (SPR) for antibody of interest as compared with members of said cell line not transformed by RHGP,

culturing said cells exhibiting a higher SPR to yield a population of cells expressing said antibody of interest at an enhanced SPR as compared with said cell line when not enhanced by RHGP.

2. The method of claim 1, wherein said altering of the expression pattern results in said at least one gene being expressed at a rate lower than the expression of said gene by said cell line without RHGP transformation.

3. The method of claim 1, wherein said altering of the expression pattern results in said at least one gene being expressed at a rate higher than the expression of said gene by said cell line without RHGP transformation.

4. The method of claim 1, wherein said cells transformed by RHGP are screened by FACS to identify a small fraction of

the transformed cells with the highest SPR for said antibody of interest to produce a screened cell collection,

said screened cell collection is expanded and subjected to a second FACS screening to identify a small fraction of screened cell collection with the highest SPR for said antibody of interest to produce a second screened cell collection,

screening said cells of said second screened cell collection by single cell plating to identify a collection of cells having a higher SPR for said antibody of interest than those of said screened cell collection or said second screened cell collection.

5. The method of claim 1, wherein said cells expressing said antibody of interest at an enhanced SPR as compared with said cell line when not transformed by RHGP exhibit an SPR at least 1.5 times that of cells of said cell line when not enhanced by RHGP.

6. The method of claim 5, wherein said cells expressing said antibody of interest at an enhanced SPR as compared with said cell line when not transformed by RHGP exhibit an SPR at least 3.0 times that of cells of said cell line when not enhanced by RHGP.

7. The method of claim 1, wherein said cell line is a Chinese Hamster Ovary (CHO) cell line, 293HEK cell line, HeLa cell line, COS cells, NIH3T3 cell line, Jurkat cell line, NS0 cell line or HUVEC cell line.

8. The method of claim 7, wherein said cell line is a CHO cell line.

* * * * *